Neuromyogenic Properties of the Internal Anal Sphincter: Applications for the Treatment of Anal Fissures

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Abstract

Pharmacological therapy of anal fissures reduces maximum resting pressure (MRP), allowing healing by promoting anodermal vascular perfusion. In this thesis the clinical and manometric responses to diltiazem and botulinum toxin and the therapeutic potential of indoramin and salbutamol were investigated. A porcine internal anal sphincter model was developed to validate the responses to pharmacological agents.

Of 33 patients with anal fissures with higher MRP than in 20 controls [110 (77-227) vs. 88 (46-175) cmH_2O, $P<0.05$], the fissures healed in 19 (58%) after a two-month course of diltiazem, [110 (77-195) vs. 81 (53-147) cmH_2O, $P<0.0001$]. Those with unhealed fissures [111 (77-227) vs. 92 (56-136) cmH_2O, $P<0.01$] received another course. At four months in 18 (75%) of 20 the fissures healed; 12 [114 (88-195) vs. 113 (51-142) cmH_2O, $P=0.07$] that had healed initially and 6 [102 (77-227) vs. 109 (47-134) cmH_2O, $P=0.50$] of 8 after a second course. At six months the fissures healed in 13 (90%) of 15 patients [110 (77-144) vs. 125 (53-161) cmH_2O, $P=0.63$]. While 58% of fissures with high MRP healed with diltiazem, 42% did not despite reduction in MRP; a subgroup with normal MRP healed although MRP remained unchanged.

A "no-needle" delivery, evaluated by injecting methylene blue in porcine anal sphincters allowed maximal instillation into the IAS at 60% to the horizontal axis, was employed to inject 25 Units botulinum toxin on either side of the fissure in 10 patients. At one week MRP [99 (71-185) vs. 81 (37-166) cmH_2O, $P<0.01$] and pain scores [6 (3-8) vs. 4 (1-8), $P<0.001$] were reduced. At three months 5 (50%) fissures healed.

Oral indoramin (20mg) reduced MRP at one hour in 10 volunteers [85 (46-136) vs. 63 (36-117) cmH_2O, $P<0.005$] and 10 patients with anal fissures [131 (94-227) vs. 88 (57-169) cmH_2O, $P<0.0001$] and the reduction was sustained for three hours. The results were similar with oral salbutamol (4mg) [94 (47-175) vs. 65 (44-101) cmH_2O, $P<0.01$] and [144 (97-194) vs. 101 (78-181) cmH_2O, $P<0.005$]. Salbutamol caused tremors in 9

subjects and was not investigated further. In 8 volunteers topical indoramin in paraffin base (10mg, 20mg, 30mg and 40mg) applying each dose on different days, like placebo had no effect after one or three hours [*P*=NS, ANOVA].

Porcine IAS muscle tissue was morphologically similar to human tissue and maintained a spontaneous tone. Histamine and phenylephrine induced concentration-dependent contractions; isoprenaline induced relaxation. Glyceryl trinitrate (GTN) at a concentration of $2.2x10^{-4}$, $6.6x10^{-4}$ and $2.2x10^{-3}$ M caused mean relaxation of 14.9%, 28.7% and 35.3%. L-NAME (N(G)-nitro-L-arginine methyl ester) abolished acetylcholine mediated relaxation due to muscarinic relaxation with nitric oxide. Adenosine triphosphate-induced relaxation was enhanced by the purinoceptor antagonist suramin. Indoramin reduced phenylephrine induced contraction suggesting an effect on α_1 adrenoceptors. Prazosin reduced histamine induced IAS contraction. Nitric oxide was the predominant but not the sole neurotransmitter for inducing relaxation, as there was some relaxation on electrical field stimulation (EFS) after the addition of L-NAME. The EFS responses in hypertonic *in vitro* porcine IAS caused by phenylephrine, were investigated to explain failure of fissures to heal with agents that reduce sphincter tone. The residual tone, i.e. the level of tone after EFS in 10 IAS strips was proportional and correlated with the level of tone before EFS, (r^2 = *0.90, P<0.001, d.f. =120*) and after GTN [$2.2x10^{-4}$, $6.6 x10^{-4}$, 2.2x10-3 M] and diltiazem [$1x10^{-4}$ M] was also proportional to the tone before treatment [r^2*=0.95, d.f.=21;* r^2*=0.87, d.f.=20;* r^2*=0.72, d.f.=20;* r^2*=0.90, d.f.=25* respectively]. Hence fissures do not heal despite reduced MRP because the ischaemic threshold, above which there is inadequate perfusion of the anoderm is not reached.

Dedication

This thesis is dedicated to my parents, Krishna and Siriniwas Bhardwaj

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Statement Of Originality

The work embodied in this thesis is the result of the author's own independent investigations, unless otherwise stated.

This work has not previously been entered or accepted for any degree or award of this or any other university. It is not being concurrently submitted in candidature for any other degree.

Work Performed By The Author

The patients and volunteers involved in every study in this thesis were recruited by the author. All anorectal physiological studies were performed by the author, who was personally responsible for maintaining contact with, and following up, all the patients and volunteers. The author was responsible for all patient consultation, preparation of patient information sheets and for keeping the general practitioners and consultants informed of their patients' progress. The author obtained ethical approval for all studies. The production pharmacists at University College Hospital prepared the test formulations. The author was directly responsible for testing these on patients and volunteers and therefore development of the various formulations.

The author was responsible for personally obtaining the animal specimens from the abattoir, and transporting them to the department of Anatomy at the University of London. The experiments outlined in this thesis were undertaken by the author, as was the interpretation of the results obtained.

In summary, apart from the preparation of the pharmaceutical agents used, all work described in this thesis was performed by the author.

Abbreviations

IAS	internal anal sphincter
EAS	external anal sphincter
MRP	maximum resting anal canal pressure
NANC	non-adrenergic non-cholinergic
RAIR	rectoanal inhibitory reflex
5-HT	5-hydroxytryptamine
ATP	adenosine-triphosphate
VIP	vasoactive intestinal peptide
NO	nitric oxide
NADPH	nicotinamide adenine dinucleotide phosphate
Ca^{2+}	calcium ion
HIV	human immunodeficiency virus
GTN	glyceryl trinitrate
Botox	botulinum neurotoxin type A
ACh	Acetylcholine

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- Neuromyogenic properties of the internal anal sphincter: therapeutic rationale for anal fissures. **R Bhardwaj**, C H V Hoyle, C J Vaizey, P B Boulos. Gut. 2000 Jun;46(6): 861-8
- The effect of indoramin and salbutamol on the internal anal sphincter. **R Bhardwaj**, C J Vaizey, P B Boulos. British Journal of Surgery, December SRS Edition 1999.
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- Topical 2% diltiazem in the treatment of chronic anal fissures. **R Bhardwaj**, CJ Vaizey, P B Boulos. Presented to the Association of Coloproctology of Great Britain and Ireland Annual meeting July 2000.
- Botulinum toxin delivered through a "no-needle" technique into the internal anal sphincter is effective in the treatment of chronic anal fissures. **R Bhardwaj**, C J Vaizey, P B Boulos. Poster presentation for the American Colorectal Society Annual meeting, San Diego. USA. 2001.
- Botulinum toxin delivered through a "no-needle" technique into the internal anal sphincter is effective in the treatment of chronic anal fissures. **R Bhardwaj**, C J Vaizey, P B Boulos. Poster presentation for the Association of Coloproctology of Great Britain and Ireland Annual meeting 2001.
- The pattern of response of chronic anal fissures to topical diltiazem treatment. **R Bhardwaj**, C J Vaizey, P B Boulos. Poster presentation to the Tripartite Colorectal Meeting, Melbourne. Australia. 2002.

CHAPTER ONE

INTRODUCTION

1.1. DEFINITION AND AETIOLOGY OF AN ANAL FISSURE

An anal fissure is a split in the skin of the distal anal canal. It is a lesion of comparatively minor pathological importance and its underlying cause remains a clinical curiosity. Young adults of both sexes are equally affected [Bennett and Goligher, 1962] and show a varied clinical presentation. Petros *et al.* (1993) in a retrospective study of 172 patients noted that anterior fissures and sentinel piles were more common in women; pruritis and rectal drainage mostly occurred in men and patients with a longer duration of symptoms and those with pruritis and drainage were more likely to have a fistula. Younger patients frequently reported rectal bleeding.

Whilst acute fissures heal spontaneously or with simple therapeutic measures a proportion progress to form a chronic linear ulcer [Lund and Scholefield, 1996]. This chronicity is perpetuated by hypertonicity of the internal anal sphincter, the underlying genesis of which is poorly understood. The difference between an acute and chronic anal fissure may be defined in both temporal and pathological terms, although the latter are more robust. The features of a chronic anal fissure include the presence of a sentinel "pile" distally, a fibroepithelial polyp at the apex, rolled edges and visible fibres of the internal anal sphincter at the base of the fissure.

Most anal fissures are idiopathic with no identifiable underlying disease process [Lund and Scholefield, 1996; Schouten *et al.*, 1996a] and there is no simple and unified theory to explain their genesis. Ball (1908) believed that the anal canal mucosa tears from a

shearing force exerted by a constipated stool leaving a linear laceration of the anoderm extending from the dentate line to the anal verge. However not all patients who develop fissures have experienced constipation [Keighley and Williams, 1993]. Ball stated the tearing down of an anal valve is important, but this is not true as an intact valve is seen at the proximal end of a fissure [Motson, 1985]. Jensen (1988) interviewed 174 patients in order to identify dietary risk factors, and noted significantly decreased risk with frequent consumption of raw fruits, vegetables, and wholegrain bread whilst an increased risk was noted with foods with little dietary fibre. Oh *et al.* (1995) investigated the aetiological factors in 1,391 patients of whom 1,313 had idiopathic fissures thought to arise from underlying inflammation. They speculated that as it was common to find stool trapped in the perianal folds, ammonia generated from the entrapped stool with chloride from sweat caused skin irritation and inflammation around the anus, and the passage of stool through the inflamed anal canal lead to tearing of the anal skin. Other aetiological factors in this study included surgery, trauma and subluxation of the anus. Whilst Oh *et al.* (1995) proposed inflammation in genesis of fissures, the progression of acute to chronic fissures was not clear. Shafik (1982) hypothesised that fissures became chronic only in patients in whom the epithelial remnants of the anorectal sinus were present in the subdermal layer of the anal canal. He proposed these remnants resulted in epithelial sequestra that harboured infection and prevented healing.

The majority of fissures occur in the posterior midline. Lock and Thomson (1977) investigated 188 patients with idiopathic fissures and noted posterior fissures in 142 (75.6%), anterior fissures in 26 (13.8%), whilst 15 (8%) had posterior and anterior

fissures and 5 (2.6%) had lateral fissures. Shafik (1982) suggested that there is lack of tissue support posteriorly within the anal canal, where a Y-shaped configuration is created by the decussation of fibres accounting for the preponderance of posterior fissures. Oh *et al.* (1995) showed that although posterior fissures are more common than anterior ones, anterior midline fissures were more common in females where the sphincter muscular support was deficient.

Current opinion regarding chronicity of fissures is based on Schouten's studies (1994, 1996 a, b, c) on the vasculature of the anoderm. Schouten *et al.* (1994) described an inverse relationship between anal pressure and anodermal blood flow. The perfusion of the anoderm at the posterior midline was significantly lower than at the sides of the anal canal. Klosterhalfen *et al.* (1989) and Schouten *et al.* (1996b) provided evidence to demonstrate that the posterior anoderm is a poorly perfused area of the anal canal, which perpetuates the chronicity of fissures although there is no firm evidence to demonstrate that ischaemia is the sole aetiological factor.

The relationship between pregnancy, childbirth and fissures is unique and is not completely understood. Abramowitz *et al.* (2002) followed 165 patients in the postpartum period and identified 25 with anal fissures; dyschezia being the most important risk factor. Whilst these patients have fissures that are commonly located anteriorly they are often associated with low anal canal pressures [Corby *et al.*, 1997]. Other causes of fissures include Crohn's disease, syphilis, human immunodeficiency virus (HIV), or

tuberculosis. These are secondary fissures and are most appropriately treated by addressing the underlying disease process.

1.2. PHYSIOLOGICAL FEATURES ASSOCIATED WITH AN ANAL FISSURE

It is now generally agreed that recognised features common to most chronic anal fissures include a high resting anal canal pressure due to hypertonicity of the internal anal sphincter (IAS), the presence of "ultraslow" pressure wave activity in the IAS, and reduced vascular perfusion index at the site of the anal fissure.

1.2.1. INTERNAL ANAL SPHINCTER ACTIVITY

Early manometric studies of patients with fissures failed to demonstrate high resting pressures. Graham-Stewart *et al.* (1961) used a large latex balloon which filled the anal canal to investigate 6 subjects and noticed unsteadiness of the pressure compared with controls and no significant difference in resting pressure. Duthie and Bennett (1964) using a manometric pull through technique with open-end water filled tubes also found no difference between patients with fissures and their controls.

Nothmann and Schuster (1974) studied 7 patients with fissures, 10 normal subjects and 7 patients with irritable bowel syndrome using a triple balloon system. The anal canal

balloon, representing the IAS tone, showed the resting pressure was twice in patients with fissures (85mmHg, range 75 to 100) than in controls (50mmHg, range 25 to 75). There was no difference in pressures in the balloon placed to represent the external anal sphincter. Hancock (1977) used a small water-filled balloon built into a hollow perspex rod and measured anal canal pressure in steps of 1cm from the anal verge in 12 patients with anal fissures. The mean maximum resting anal pressure in patients with anal fissures (116.8 ± 21.8, s.d., cmH_2O) was significantly higher than in controls (85.0 ± 20.5, s.d., cmH_2O; $P<0.01$).

The discrepancies of earlier studies that failed to identify raised anal canal pressure could in part be explained by the recording methods used. Graham-Stewart *et al.* (1961) failed to get steady readings in patients with fissures, and Duthie and Bennett (1964) may have not used a sufficient perfusion pressure in their recording equipment. Hancock (1976) had shown that in a fine perfused recording system the speed of perfusion does influence the pressure and motility pattern recorded. Gibbons and Read (1986) attempted to eliminate recording bias by investigating the effects of different diameters of probes on resting anal canal pressure. The results in 6 patients with fissures exceeded the normal range as compared with 14 controls irrespective of the probe size. With the smallest probe (0.4cm diameter) the resting pressure in patients was 114 ± 17.1 cmH_2O (mean, s.d.) compared to 73.1 ± 27.0 cmH_2O in controls (mean, s.d.). These results suggested that high resting pressures were recordable in patients with fissures even when small probes were used and were unlikely to be due to spasm; therefore they probably represented a true increase in basal sphincter tone. Lin (1989) examined resting,

voluntary contraction pressures and sphincter length in 29 patients with chronic anal fissures, 50 patients with Grade III or IV haemorrhoids and 36 controls with no anorectal symptoms or pathology. The maximal basal pressures in chronic anal fissures, haemorrhoids, and controls were 87.4 ± 38.8, 85.3 ± 27.7, and 71.2 ± 24.9 mmHg; the maximal contraction pressures were 162.1 ± 64.5, 158.8 ± 58.0, and 132.9 ± 44.9 mmHg; the lengths of the functional sphincter of the three groups were 3.9 ± 0.6, 3.8 ± 0.8, and 3.7 ± 0.5 cm. The maximal basal pressures and maximal contraction pressures in patients with fissures or haemorrhoids were significantly greater than in the control group; whereas the functional sphincter lengths in the three groups showed no significant difference.

Hancock (1977) noted that IAS activity in the presence of fissures was abnormal. He classified the motility pattern of the IAS during recording into one of three groups: *Flat* – steady anal pressure showing only slow waves; *Irregular* – resting pressure unsteady, sometimes with marked fluctuations in pressure; *Ultra-slow waves* – regular pressure waves of amplitude greater than 25 cmH_2O and frequency less than 2/min. In the 12 patients with fissures examined ultraslow pressure waves were present in 10, but only in 2 of 40 controls ($P<0.001$). These waves were thought to represent the chronic overactivity of the IAS in association with a fissure. Schouten and Blankensteijn (1992) investigated these ultra-slow waves and demonstrated these waves were present in 29 of 58 patients with fissures and in 2 of 20 controls. The median value of maximum anal canal pressure in those patients and volunteers with ultra-slow waves was higher than those subjects who did not (158 v 138 cmH_2O, $P<0.05$ and 181.5 v 92 cmH_2O, $P<0.001$

respectively). Two weeks after surgical treatment these ultra-slow waves had disappeared in half of the patients, with a reduction in maximum anal canal pressure to the levels in control subjects without these waves. The pressure reduction was significantly greater than in patients with persistent ultra-slow waves (40% v 15%, $P<0.02$). The authors concluded that these waves were the manifestation of increased activity of the IAS. Keck *et al.* (1995) also demonstrated ultraslow wave activity in 11 of 12 patients with chronic anal fissures and showed greater amplitude of ultra-slow wave activity as compared with controls (31 mmHg v 15 mmHg). They also investigated the high pressure zone/ sphincter length ratio in patients and controls and showed this ratio was 58% compared with 48% in controls. Interestingly the elevated pressure was not confined to the site of the fissure; this led the authors to conclude that the primary abnormality in fissures is persistent hypertonia affecting the entire internal sphincter.

Farouk *et al.* (1994) investigated internal sphincter activity in 30 patients with anal fissures, 22 patients with haemorrhoids and 33 controls with ambulatory anal sphincter fine-needle electromyography and anorectal manometry. The median internal sphincter electromyography frequency was similar in the fissure group, the haemorrhoid group and the control group (0.49 Hz, 0.46 Hz ($P>0.05$), and 0.44 Hz ($P>0.05$) respectively). The median anal resting pressures were similar in the fissure group (132 cmH_2O) and the haemorrhoid group (116 cmH_2O, $P>0.05$), but significantly greater than in the control group (94 cmH_2O, $P<0.05$). The median number of transient relaxations of the internal anal sphincter with an associated rise in rectal pressure was 1 (range, 0-4) per hour in the fissure group, 6 (range, 4-7) per hour in the haemorrhoid group, and 4 (range, 3-6) per

hour in the control group. The investigators were able to only investigate 6 patients with fissures following lateral internal sphincterotomy, between 12 and 18 months postoperatively. All these fissures had healed. The median anal canal pressure was 102 cm of H_2O (P>0.1 vs. controls) and the number of internal sphincter relaxations increased to 4 per hour ($P < 0.01$ vs. preoperative number). The authors concluded that IAS relaxation was less frequent in patients with chronic anal fissures that have failed to heal in comparison to patients with haemorrhoids and normal controls. This evidence further supported the hypothesis that internal sphincter hypertonia may be relevant to the pathogenesis of this disorder.

Kuypers (1983) and later Jost *et al.* (1995a) have shown the IAS in healthy volunteers relaxed in response to rectal distension; the recto-anal inhibitory reflex. Nothmann and Schuster (1974) recorded abnormalities of IAS reflex effects in response to rectal distension. Whilst there was an initial relaxant response as in controls, in 90% patients with fissures as compared with 26% of controls a rebound or "overshoot" contraction was noted. There were no changes seen in the external anal sphincter at the time of this "overshoot". The significance of this abnormal response of the IAS was unclear at this stage. However this was not a consistent finding as Keck *et al.* (1995) demonstrated in 12 patients with chronic anal fissures a normal rectoanal reflex without "overshoot". Reissman *et al.* (1995) evaluated this "overshoot" phenomenon in 37 HIV +ve patients, 28 of whom were homosexual, 9 who were bisexual, and 7 control HIV -ve patients. It was not stated whether the control patients were homosexual or bisexual. All had a single posterior fissure except 6 of the 37 HIV +ve patients who had multiple and/or

lateral fissures. The "overshoot" was absent in 35 (95%) of 37 of HIV +ve patients but was present in all HIV –ve patients. The aetiology in 19 patients was determined to be due to HIV, cytomegalovirus, herpes simplex virus or from a leukaemic infiltrate. Two patients who were HIV +ve and demonstrated the "overshoot" contraction benefited from division of the IAS. In the remaining 16 patients no aetiological factors were identified and were treated conservatively with debridement, and anal hygiene. The authors concluded that the majority of fissures noted in HIV +ve patients are not associated with IAS hypertonia or overshoot contraction of the IAS in response to rectal distension. After infections have been excluded patients should undergo anorectal manometry to determine the truly benign fissures; these may benefit from surgery.

1.2.2. ISCHAEMIC NATURE OF ANAL FISSURES

Klosterhafen *et al.* (1989) conducted postmortem angiographical analyses of the vasculature of the posterior commissure of the anal canal. They demonstrated in 85% of nonselected autopsies that the posterior commissure was the end of the capillary system of the inferior rectal artery. Schouten *et al.* (1994) demonstrated an inverse relationship between anal pressure and blood flow. They performed doppler laser flowmetry of the anoderm combined with anorectal manometry in 178 subjects, consisting of 31 healthy volunteers, 23 with fecal incontinence, 17 with haemorrhoids, 9 with anal fissure, and 98 with other colorectal disorders. They examined anodermal blood flow, flux represented as V, in the four quadrants of the anal canal in 16 controls and showed that perfusion of the anoderm at the posterior midline was significantly lower than in the other three

segments (posterior midline: 0.74 ± 0.26 V; left lateral side: 1.68 ± 0.81 V; right lateral side: 1.57 ± 0.52 V; anterior midline: 1.48 ± 0.69 V, $P<0.001$). Overall they found a significant inverse correlation between maximum anal resting pressure and anodermal blood flow at the posterior midline ($r = -0.616$, $P<0.001$). In the 9 patients with chronic anal fissure, the mean maximum anal resting pressure was 125 ± 26 mmHg, which was significantly higher than in patients with haemorrhoids (82 ± 15 mmHg), controls (66 ± 19 mmHg), and patients with fecal incontinence (42 ± 14 mmHg, $P<0.001$), whereas the blood flow at the base of the fissure was significantly lower (0.43 ± 0.10 V v 0.57 ± 0.19 V vs. 0.75 ± 0.26 v 1.03 ± 0.34 V). They also demonstrated in 10 patients that during anaesthesia the anal pressure dropped from 63 ± 21 mmHg to 32 ± 15 mmHg ($P < 0.001$), whereas the anodermal blood flow at the posterior midline increased from 0.79 ± 0.22 V to 1.31 ± 0.35 V ($P<0.001$). The authors concluded that anodermal blood flow at the posterior midline was less than in the other segments of the anal canal and the perfusion of the anoderm at the posterior commissure was strongly related to anal pressure. The higher the pressure, the lower the flow. These conclusions led Schouten *et al.* (1996b) to propose that chronic anal fissures were ischaemic ulcers. These ideas were further investigated in a recent postmortem topographical count of arteriole density in anal canals from 8 cadavers by Lund *et al.* (1999). They showed a paucity of vessels in the posterior quadrant. This helped to bolster Schouten's ideas on the ischaemic nature of anal fissures.

1.2.3. THE EXTERNAL ANAL SPHINCTER

Few studies have investigated the role of the external anal sphincter in the pathogenesis or treatment of chronic anal fissures. Nothmann and Schuster (1974) showed no abnormal manometry from a balloon placed to measure the pressure of subcutaneous external anal sphincter. Gibbons and Read (1986) also demonstrated no significant difference in maximum squeeze pressure between 6 patients with fissures and 14 controls (272.8 ± 92.2 v 284.8 ± 59.5 cmH_2O). However Jost *et al.* (1995a) investigating the external anal sphincter electromyographically found in 9 of 12 patients studied there was overactivity of the external anal sphincter and argued that as a contribution of the resting anal pressure arose from the external sphincter treatment directed against this muscle was also necessary.

1.3. HISTOPATHOLOGY OF THE INTERNAL SPHINCTER IN AN ANAL FISSURE

Miles (1919) in his anatomical studies of haemorrhoids described an area of circular fibrosis at the lower end of the anal canal that he coined with the term "pecten band" that was not present in a healthy anal canal. He failed to recognise this band as the IAS fibres and felt that division of this band was necessary for treatment. We now know that a hypertonic IAS is associated with both internal haemorrhoids and fissures and as such this "pecten band" is present in both conditions. The "pecten" was initially described by Stroud (1896) as an area in the middle third of the anal canal and marked the junction of the proctodeum and the hind gut. Abel (1932) sought to clarify the histological nature of

the "pecten band" associated with anal disease. He agreed that hypertonicity or spasm occurring in the alimentary canal produced local passive congestion, which resulted in fibrous tissue deposition and that pectenotomy was a curative procedure for treatment of this band, but failed to recognise that this band was IAS fibres. Fine and Laws (1940) demonstrated the presence of smooth muscle fibres only in biopsies from the region in normal volunteers. It was Goligher (1961) who realised that the pecten band was the IAS. Petrozzi *et al.* (1967) histologically analysed tissue from 24 resected anal fissures and noted loss of substance in the mucosa and in the deeper layers, with an intense chronic inflammatory process and acute activity. Brown *et al.* (1989) sought to clarify the histopathology of the IAS in patients with chronic anal fissures. In all 18 patients studied fibrosis was evident in the IAS compared with 4 control specimens, and in 16 fibrosis was more prominent in the base of the fissure rather than laterally in the IAS. The authors suggested that the myositis that is associated with chronic anal fissures may explain the spasm and fibrosis.

1.4. TREATMENT OF ANAL FISSURES: CONSERVATIVE MEASURES

The initial approach in the treatment of anal fissures is non-operative. Sharp (1996) suggested that an acute anal fissure will heal spontaneously or in response to medical therapy with warm baths, stool softeners, bulk laxatives, analgesics, topical anaesthetics and reassurance. The severe, constant, commanding pain of a chronic fissure is a more difficult to treat. In order to treat the initiating event in the development of fissures, the

avoidance of constipation has been emphasised. Jensen (1986, 1988) found that consumption of certain foods increased the risk of developing a fissure, and that dietary bran supplements and warm sitz baths were superior to topically applied local anaesthetic or hydrocortisone cream. Jensen (1987) also investigated the maintenance therapy with unprocessed bran in the prevention of acute anal fissure recurrence. Patients were randomised to either receive placebo, 2.5mg or 5g thrice daily for one year. The recurrence rates were 68%, 60% and 16% respectively, and significantly fewer recurrences occurred in patients receiving higher dose of dietry bran.

Fries and Reitz (1964) investigated whether medical therapy should be reserved for anal fissures of short duration. In 18 patients with fissures of 3 months or less duration healing occurred in 9 (50%), whilst in 61 patients with a mean duration of symptoms of 26.4 months the healing rate was 39.3%. The difference was not significant to reserve conservative for fissures of short history only. They reinforced the notion that the duration of symptoms did not influence the healing rate; as fissures healed in 15 (38.5%) of 39 patients with a history less than 6 months compared with 18 (45%) of 40 with symptoms of more than 6 months duration.

Gabriel (1930) recommended practice of regular anal dilatation for the treatment of anal fissures. Lock and Thomson (1977) conducted a study in 133 patients who were treated with anal dilatation and topical local anaesthetic gel. Healing occurred in 71 (53.4%) who were then discharged from further attendance. 58 of these healed patients replied to long-term follow-up questionnaires and 33 (56.9%) remained symptom free. The

presence of a sentinel tag or fibrous anal polyp was the only significant predictor of failure to respond to conservative treatment. Gough and Lewis (1983) established a randomised clinical trial in 82 patients to assess the value of regular anal dilatation in combination with topical local anaesthetic. After 1 month's treatment 17 (43.6%) of 39 in the lignocaine gel group had healed compared with 18 (41.9%) of 43 in the group that used lignocaine gel and anal dilatation. There was no significant difference in these treatment modalities and anal dilatation could therefore not be advocated. However they found that a sentinel tag was present in 18 (54.5%) of 33 whose fissures healed on conservative therapy compared with 23 (50%) of 46 whose fissure persisted despite treatment. Furthermore they suggested abandoning the use of a dilator in favour of topical local anaesthetic agents and early lateral sphincterotomy.

McDonald *et al.* (1983) randomised 81 patients with acute anal fissures for treatment with either lignocaine gel and "Normacol" granules or an anal dilator. Of those that returned for follow up at 6 weeks 11 (31.4%) in the group that used the dilator compared with 12 (38.7%) that did not had failed treatment. This difference was not statistically significant and reinforced the idea that an anal dilator was of no added value to medical therapy.

Other simple therapeutic measures such as silver nitrate cautery to the fissure, sitz baths, and anal dilators have been disappointing. Shub *et al.* (1978) studied 393 patients treated with cautery, suppositories and sitz baths; 44.4% of healed in the short term but 27.2% developed recurrent anal fissures over a 5-year follow up period.

1.5. TREATMENT OF ANAL FISSURES: OPERATIVE STRATEGIES

"Of all structures in the area, one stands out as the king. You can damage, deform, ruin, remove, abuse, amputate, maim or mutilate every structure in and around the anus except one. The structure is the sphincter ani" [Bornemeier, 1960].

Current treatment has aimed at reducing resting anal pressure by diminishing sphincter tone and improving blood supply at the site of the fissure, thus promoting the healing rate. Hughes (1953) acknowledged that anal spasm prevented the fissure from healing, though he mistakenly stated that the base of the fissure was formed by the circular fibres of the subcutaneous portion of the external anal sphincter. We know now that these fibres are of the internal anal sphincter.

Early operative techniques for the treatment of fissures included lateral sphincterotomy (Boyer, 1818), the midline posterior sphincterotomy (Dupytren, 1833), excision of the fissure with a triangular piece of perianal skin (Gabriel, 1930), and the simplest of all an anal stretch (Récamier, 1838). Goodsall (1892) stated that open anal sphincterotomy through the base or near the fissure "invariably cured the fissure". An early report by Martin (1922) recommending subcutaneous sphincterotomy performed blindly in the posterior quadrant was not received well. Miles's (1939) "pectenotomy", that is the division of what he called the pecten band in the lower part of the anal canal, was in fact internal anal sphincterotomy as the work by Eisenhammer (1951) and Goligher (1956)

had shown this band to be the lower fibres of the internal sphincter. The word pecten arises from the latin translation of "a comb".

Eisenhammer (1951) introduced the term "chronic anal contracture" with the misbelief that the chronic fissure syndrome became fully established only when the IAS underwent changes in contractility. Nevertheless he recognised the failure of anal dilators in treating anal fissures, and reintroduced the procedure of "linear internal anal sphincterotomy", aware of the associated risk of incontinence. In his method sphincterotomy was combined with anal dilatation with an anal dilator (diameter of 1 7/8 inches) and excision of the fissure.

1.5.1. ANAL DILATATION

Manual dilatation of the anus is a simple procedure and previously a popular treatment option. Récamier first described it for the management of anal fissure in 1838. Its reintroduction by Goligher (1961) and its popularisation by Lord (1968) for third-degree haemorrhoids led to its acceptance. Watts *et al.* (1964) in an early comparison between dilatation and internal sphincterotomy recommended the former technique as the first line treatment. In MacDonald *et al.'s* (1992) group of 46 patients treated by anal dilatation the fissures failed to heal in 26 (56.5%); 2 patients experienced major incontinence, whilst 9 experienced minor incontinence. The advent of endoanal ultrasonography provided an insight as to the degree of damage associated with this procedure. In 12 men with faecal incontinence with a mean resting anal canal pressure of 49 ± 3 cmH_2O (mean

± s.e.m.) Speakman *et al.* (1991) demonstrated by ultrasonography internal anal sphincter defects in 11 with a mean fragmentation of 153^0 of the circumference. There were also external anal sphincter defects in 3 patients. Nielsen *et al.* (1993) demonstrated the risk of sphincter damage and anal incontinence in a study of 32 patients with a median follow up of 4 years (range 2 to 6 years). Anal dilatation resulted in incontinence in 12.5% of patients, although sphincteric defect was evident in 65% of 20 patients who underwent endoanal endosonography. Strugnell *et al.* (1999) advocated gentle digital dilation of the anus (DDA) under total neuromuscular blockade. Over a 15-year period 273 who underwent DDA were followed up (median 7.8 years) by a questionnaire or telephone interview; none were incontinent; 15 patients had impaired control, 9 (3.8%) followed and 6 preceded the treatment. Twenty-three patients in this study who had experienced either temporary or permanent impairment, whether or not pre-existing, were invited to attend for ultrasonography and manometry. Of 18 examined no sphincteric damage was seen, and the manometric studies were normal. The authors advocated care when performed by an unsupervised trainee.

1.5.2. INTERNAL ANAL SPHINCTEROTOMY

Introduction in the Twentieth Century

Eisenhammer (1951) had coined the term "chronic anal contracture" with certain anal conditions and for treatment of fissures recommended a 4/5 division of the muscle, and

total division extending to the circular muscle of the rectum when there was pronounced contracture.

By 1959 the "standard internal sphincterotomy", as described by Eisenhammer had been modified to comprise division of only half of the IAS to the dentate line in its lateral or posterolateral part. Further division was performed when proximal relaxation of the sphincters was required; and complete division of the IAS was deemed necessary in cases where pain and spasm were pronounced. Brossy (1965) believed that the chronicity of anal fissures was maintained by chronic spasm or contracture of the internal anal sphincter and advocated a complete sphincterotomy. Eisenhammer (1959) further proposed excision of the hypertrophied papillary polyp above and the external tag (sentinel pile) below the fissure and undermining the edges of the fissure to divide the tethering fibres. However, it was recognised that secondary chronic anal fissures were often associated with normal sphincter tone, and it was imperative to avoid division of the IAS in these cases. Quite correctly, Eisenhammer (1959) emphasised a degree of conservatism regarding surgery of the anal canal.

Recognition of postoperative functional results

Although internal sphincterotomy was recommended by Morgan and Thompson (1956), Lockhart-Mummery (1957) and Goligher (1961); Bennett and Goligher (1962) recognised the lack of information on the subsequent effect on anal function. Morgan and Thompson (1956) never commented on the functional result in their patients treated by

sphincterotomy. Goligher (1961) demonstrated that although some patients developed impaired control for several weeks eventually anal function returned to normal. Bennett and Goligher (1962) reviewed 127 cases of anal fissure treated by sphincterotomy after 3 months to 6 years. They noted that continence was defective frequently in the first few weeks after operation (34% for flatus, and 15% for faeces). This gradually improved although at time of interview there was still evidence of defective function (24% for flatus, 11% for faeces and 28% had faecal soiling). Whilst the median follow up time was not stated 105 cases had been followed for a year and 23 for more than 3 years. There was no clear correlation between functional disturbance and the extent of internal sphincter division, estimated by reference to the operation notes, and by palpation of the gap at the time of review, although only 20 patients had complete sphincterotomies. The recurrence rate in the series of 127, measured by recurrence of pain was 7%. It appeared that the recurrences occurred after one year. Interestingly Brossy (1965) claimed no lasting defect of continence with complete division of the sphincter.

Dilatation vs. sphincterotomy

Watts *et al.* (1964) compared prolonged (minimum four minutes) dilatation of the anus with lateral sphincterotomy in 241 patients (141 patients had lateral sphincterotomy, and had been previously reported by Bennett and Goligher (1962)). Follow up was available for 95 patients; anal dilatation produced rapid relief of pain, but with symptomatic recurrence in 16%. Defects in anal control, which comprised of either incontinence to flatus or faeces or soiling, occurred in 28 (31%) of 90 with dilatation as compared with

43 (34%) of 127 after sphincterotomy. The authors concluded that anal dilatation was the procedure of choice and recommended internal anal sphincterotomy in the event of recurrence.

Outpatient sphincterotomy

Magee and Thompson (1966) reviewed the results of 139 patients who underwent internal anal sphincterotomy as an outpatient procedure with xylocaine local anaesthesia between 1962 and 1963. In a posterior or posterolateral fissure the muscle incision was at the base of the fissure, but was made in the left posterolateral quadrant for an anterior fissure. The operation was successful in the majority, but 3% of patients had recurrent fissures on examination, and another 9% had pain on defaecation when the stool was hard. Imperfect control of faeces and flatus was noted in 3% and 17% respectively, and 41% of patients complained of soiling of their underwear. These results were comparable to later reports [Khubchandani *et al.*, 1989; Oh *et al.*, 1995].

Effects of posterior sphincterotomy

Hardy (1967) reviewed the results of posterior sphincterotomy in 59 patients. There were no recurrences but 9 (15%) patients had some form of incontinence, and 30 (51%) complained of some mucous discharge. On examination, 15 patients had a "keyhole deformity" posteriorly that was regarded as the cause of mucous discharge, of whom 9 complained about. Hawley (1969) randomly allocated 18, 32 and 24 patients to anal

stretch, posterior sphincterotomy or a lateral sphincterotomy respectively. The fissures healed in all patients who underwent lateral sphincterotomy, in 30 (92%) who had a posterior sphincterotomy and in 13 (72%) after anal stretch. The two patients that experienced soiling had a posterior sphincterotomy.

The lateral internal anal sphincterotomy

Parks (1967) in refining the technique of lateral sphincterotomy described dividing the lower part of the internal sphincter under vision through a short circumferential incision in the skin at the lateral anal verge that was closed by suturing. Notaras (1969) is credited for promoting the technique of lateral subcutaneous internal sphincterotomy that Riberio (1960) described initially in 112 patients. In this technique the lower part of the internal sphincter is divided as in performing a subcutaneous tenotomy by introducing the knife blade at the anal verge between the anal canal mucosa and the IAS, then directing the cutting edge laterally towards the IAS. Notaras treated 66 patients with immediate relief of pain and the fissures healed by 3 weeks. The incidence of faecal soiling was 6%, compared to 30% and 41% following posterior internal sphincterotomy reported by Bennett and Goligher (1962) and Magee and Thompson (1966) respectively. Hoffman and Goligher (1970) reviewed 99 patients who underwent lateral subcutaneous sphincterotomy, with a mean follow up period of 11 (range 3 to 24) months. The technique involved passing the blade between the internal and external sphincters and cutting medially, which differed from Notaras' technique where the IAS was divided from within outwards. One patient had a brisk reactionary haemorrhage, and another

developed a perianal abscess and subsequent fistula at the site of sphincterotomy. All but 4 healed by one month. There were 3 (3%) recurrences; one had a further internal sphincterotomy, and the other two though completely relieved of anal pain, had persistently unhealed anal fissures. Twelve (12%) patients had impaired continence and/or soiling. Millar (1971) by incising the lower two-thirds of the internal sphincter in a series of 99 patients healed over 80% within two-weeks with no evidence of recurrences noted at a follow up period ranging from 3 months to 4 years. He concluded that at that time lateral anal sphincterotomy for chronic anal fissure was deemed to be the best available treatment.

Which technique of sphincterotomy?

Although there had not been a formal randomised clinical trial comparing the various operative techniques lateral internal sphincterotomy was adopted in preference to anal dilatation and posterior sphincterotomy. This was a pivotal point in the surgical management of anal fissures, as studies then sought to identify several important factors. These included either improvement in the operative technique or comparing outcome measured by rate of complications, resolution of symptoms, healing and recurrence rates, functional impairment and effect on sphincter pressure.

Notaras (1971) treated 82 patients by lateral sphincterotomy. All fissures but 2 (2.4%) healed by 3 weeks. At 6 months in a postal survey those that replied, 90% of the total group were pain free. Of 73, 4 (5.5%) patients complained of soiling of their

underclothes 3 weeks after the operation and continued to complain of this symptom at 6 months. Rudd (1975) carried out open lateral subcutaneous sphincterotomy with the patient in the prone jack-knife position, and under lidocaine local anaesthesia the IAS was divided with the radial skin incision left open. In a follow up of 3 to 24 months, with an undefined median period, there were no recurrences. The fissures healed in all 200 patients except one, who subsequently was diagnosed with Crohn's disease. No patient developed incontinence to flatus or stool. Bailey *et al.* (1978) investigated 418 patients with anal fissures, some with associated anal disease, who underwent open lateral sphincterotomies with a radial incision over a 3 year period. Complications occurred in 22 (5.3%) patients that included urinary retention (0.2%), bleeding (1%), abscess formation (0.7%), fistulae in the sphincterotomy site (1.2%), and incontinence to gas or faeces (2.2%) that was minor in all but one who required a sphincteroplasty. Ravikumar *et al.* (1982) carried out subcutaneous lateral sphincterotomy in 60 patients, 45 (75%) of whom had posterior fissures. In 40 (67%) the fissures healed within 10 days, in 18 (30%) within 10 to 20 days, in the remaining 2 (3.3%) by 5 weeks, and 55 (91.7%) returned to work within 48 hours. Only 3 (5%) patients had minor soiling that resolved within three weeks. During a minimum of two years follow up there were no complications or recurrences. The authors suggested several operative guidelines. Firstly the sphincterotomy should be away from the fissure site so that intact mucosal bridges fill the gap between divided muscle fibres to allow rapid healing. Secondly the entire thickness of the lower internal sphincter must be divided, as any remaining intact fibres go into intense spasm to compensate for the divided fibres. Thirdly the upper one-third of the sphincter must remain intact for continence. Finally the mucosa over the sphincterotomy

site should not be breached as this would predispose to infection. While excision of the sentinel pile was not addressed the authors observed that small skin tags regressed completely and larger ones regressed considerably after sphincterotomy and none of the patients treated wished to have the sentinel tag excised at a later date.

Whilst lateral subcutaneous sphincterotomy was increasingly accepted the recurrence rate when performed under local anaesthesia was of concern. Marby *et al.* (1979) reported a recurrence rate in 13 (29%) patients as compared to 4 (10%) patients in a group of 156 treated by either subcutaneous sphincterotomy under local anaesthesia or anal dilatation under general anaesthesia. In those patients with recurrence, the maximum resting pressure (MRP) remained unchanged. Keighley *et al.* (1981) treated 71 patients with acute anal fissures by lateral sphincterotomy under either local or general anaesthesia. Although at one month the majority of patients were pain free, at four months the clinical picture changed considerably; in 14 (41%) of 34 patients who were treated under local anaesthetic the fissures recurred. All the fissures healed in the group treated under general anaesthesia. At four months the reduction (21%) in MRP was greater in patients treated under general anaesthesia (131 ± 25 to 104 ± 36 cmH_2O, n=6) than the 13% reduction in those treated under local anaesthesia (124 ± 34 to 109 ± 31 cmH_2O, n=5), but the numbers were too small to demonstrate statistical significance.

Boulos and Araujo (1984) investigated the adequacy of sphincterotomy by either a subcutaneous or an open technique in 28 patients with chronic anal fissures. Both groups were relieved of pain within a week, and the fissure healed in all subjects at one month.

The reduction in MRP after subcutaneous sphincterotomy was 51%, and 50% after open sphincterotomy, with incontinence to flatus in 3 (21%) and 2 (14%) respectively, but on further follow up these patients regained full control. Complications included skin ecchymosis and discolouration at the site of the wound, particularly after subcutaneous sphincterotomy. One patient in each group developed sepsis, which did not require treatment. This study demonstrated that the extent of sphincter division and the resultant reduction in MRP was similar, and the subcutaneous sphincterotomy was of comparable efficacy to open sphincterotomy. The authors concluded that although the subcutaneous technique was similar, the open technique allowed better control of bleeding.

Long term effects of sphincterotomy

Chowcat *et al.* (1986) examined the long-term effect of either open or subcutaneous technique on MRP in 28 patients with anal fissures. Preoperatively the median MRP in patients with anal fissures was 98.0 cmH_2O, which was significantly higher than the median MRP of 72.6 cmH_2O in 14 control patients. The reduction in MRP that was maintained at 12 months was about 50%. Cerdan *et al.* (1982) studied 15 patients whose MRP was 95 cm H_2O, which was significantly higher than in controls, and was reduced to a normal anal canal pressure after sphincterotomy. Although MRP was reduced after sphincterotomy [Boulos and Araujo, 1984], Melange *et al.* (1992) were unable to demonstrate a correlation between preoperative and postoperative clinical symptoms and manometry. Garcia-Aguilar *et al.* (1996) analysed the data of 324 patients who underwent open sphincterotomy; and 225 patients who underwent subcutaneous sphincterotomy, with an average follow up of 36

months. Statistically significant differences were noted in difficulty in controlling flatus (30.3% vs. 23.6%), soiling (26.7% vs. 16.1%), and accidental bowel movements (11.8% vs. 3.1%). When patients were asked to express their degree of satisfaction 90% reported overall satisfaction, fewer patients who had undergone the open sphincterotomy than those who had closed technique were satisfied (49.7% vs. 64.4%). However, similar numbers of patients (1.2% vs. 1.3%) were very dissatisfied. The authors concluded that, although open and subcutaneous internal sphincterotomy were equally effective in controlling the symptoms of chronic fissures, the closed technique was preferable because it demonstrated less impairment of continence.

Although internal sphincterotomy allowed adequate division of the IAS with minimal morbidity, a high cure rate and low recurrence rate the long term sequelae were not addressed. Walker *et al.* (1985) conducted a retrospective review of 306 patients who underwent either closed or open sphincterotomy, alone or with other anal procedures including haemorrhoidectomy or fissurectomy. The follow up period ranged from three to five years (mean 4.3 years). They demonstrated that, although all patients were cured of anal fissure or stenosis, complications were observed in 110 (36%) patients. The complications after closed sphincterotomy alone occurred in 10 (22%) of 45 patients, after open sphincterotomy alone in 21 (55%) of 38 patients and in 79 (35.4%) of the remaining 223 patients who had sphincterotomies combined with other procedures.

Though the authors did not state the period of short-term follow up, they indicated that that short term complications included an unhealed wound, pruritis, pain, incontinence to flatus, and bleeding. Among the 100 patients available for long term follow up (mean 4.3 years (range 3 to 5 years)) 9 were incontinent to flatus, 6 complained of minor faecal soiling, 5 reported anal discharge and 4 complained of urgency. When different procedures were compared, closed sphincterotomy had fewer complications; though this comparison was without statistical qualification. The authors felt that patients ought to be warned of possible minor complications and impairment of continence. Lewis *et al.* (1988) in an analysis of 247 closed and 103 open sphincterotomies reported a healing rate of 95.4% with an incidence of incontinence of 6.6% that was slightly higher after the closed 6.9% than the open 5.8% sphincterotomy. The minimum follow up period was 14 months (mean 37 months).

The largest review of the sequelae of internal sphincterotomy for chronic fissure in ano involved 1355 patients, the majority of whom underwent open bilateral sphincterotomy. The follow up ranged from four to nine years [Khubchandani and Reed, 1989]. Of the 1057 patients whose outcomes were recorded in the case notes the fissure healed in 1033 (97.7%) patients. Responses were received from 829 (61.2%) patients and 1102 (81.3%) physicians, with a common subset of 717 (52.9%) subjects. Flatus incontinence, occurring "sometimes" to "infrequently" occurred in 255 (35.7%) of 715 subjects and was not related to gender. An alarming 35 (4.9%) of 716 complained of faecal urgency, whilst 152 (21.2%) of 716 described soiling. There were statistically no differences between the method of sphincterotomy an terms of incontinence to flatus, soiling or

urgency. Only 56 patients were not satisfied with the outcome of their surgery. In a similar questionnaire survey by Nyam and Pemberton (1999) 10 years later showed that in 487 of 585 who responded whose mean follow up was 72 months the healing rate was 96% and 98% of the patients were satisfied with the result. Episodes of faecal incontinence occurred in 219 (45%) of 487. Females were statistically significantly more prone to flatus incontinence and though males complained of soiling, this did not reach statistical significance. Only 3% of patients felt that incontinence affected their quality of life.

Pernikoff (1994) reappraised the technique of lateral internal sphincterotomy by examining the results, the morbidity and mortality in a retrospective review of 290 patients who had an open sphincterotomy and 210 who had a closed sphincterotomy, with a mean follow up of 5.6 (range 1-11) years. Only 1% failed to heal. Long-term minor complications occurred in 55 (11%) patients. These included minor faecal soilage in 22 (4.4%), incontinence to flatus in 14 (2.8%), bleeding in 7 (1.4%), urgency in 5 (1%) and pain in 10 (0.2%). Complications were noted in 43 (15%) of 290 of those who had an open sphincterotomy group compared with 17 (8%) of 210 in the closed sphincterotomy group; the incidence of complications in the former group were significantly greater. Sharp (1996) felt that a follow up of less than 10 years was unlikely to identify cumulative causal effects on incontinence.

Littlejohn and Newstead (1997) showed that when sphincterotomy was extended to the dentate line incontinence rates ranged from 0-35 %, with a healing rate of 93-100%, and a

recurrence rate of 0-25%. They advocated a "tailored sphincterotomy" where the sphincter was divided to the length of the fissure. The healing was 99.65%, with complete resolution of symptoms in those that required a repeat sphincterotomy. Complications included one patient with faecal soiling (0.35%), four patients with flatus incontinence (1.4%), and two with faecal urgency (0.7%).

1.5.3. FISSURECTOMY/ FISSURECTOMY AND SPHINCTEROTOMY

Gabriel (1930) made a square incision around the fissure with removal of any associated tags allowing drainage a fissure. The wound was left open. Though this was not a true fissurectomy the purpose of the procedure was to drain a deep ulcerated posterior fissure. No mention was made of dividing the IAS.

Hughes (1953) believed that the radical cure of an anal fissure entailed excision of the fissure, division of the subcutaneous sphincter muscle and application of a split skin graft to the area as to shorten convalescence. In his series 12 patients following excision of the fissure combined with primary skin grafting had a 100% "take" and left hospital within a week.

Abcarian (1980) compared 150 patients who had lateral internal sphincterotomy with an equal number who had fissurectomy-midline sphincterotomy. The fissurectomy was performed by making a 2cm U-shaped midline incision at the site of the fissure separating the skin from the external sphincter and dissecting the anal fissure from the scarred

internal anal sphincter. The fissure and its accompanying abnormalities (i.e. hypertrophic anal papilla and sentinel pile) were then excised to the dentate line and a superficial midline sphincterotomy was made in the distal half of the internal anal sphincter. Finally, the cut edge of the rectal mucosa was sutured to the internal sphincter at the area of the dentate line. It was found that hospital stay (3-4 vs. 1-2 days), time for resolution of symptoms (2-3 weeks vs. 1-2 weeks) and wound healing (6-7 vs. 2-3 weeks) were doubled following fissurectomy-midline sphincterotomy compared to lateral sphincterotomy. Early and temporary loss of continence for flatus was fairly common in both groups (40% fissurectomy-sphincterotomy vs. 30% internal sphincterotomy). After two weeks these problems resolved in those following internal sphincterotomy, however occasional loss of flatus and faecal soilage were seen in 8 (5%) patients among the other group. This was attributed to a "key-hole" deformity due to scarring leaving a permanent gutter at the anal verge. In each group 2 (1.3%) recurred. Abcarian concluded although lateral internal sphincterotomy was the procedure of choice because of low risk of incontinence, fissurectomy had a role in midline fissures complicated by underlying fistula.

Bode *et al.* (1984) reviewed 121 patients who underwent fissurectomy and midline sphincterotomy with a mean follow-up of 8.1 years. Minor incontinence occurred in 30 (25%) patients in the immediate postoperative period that resolved by two months. Of 6 (5.0%) recurrences, 5 (4.1%) were acute fissures in the fissurectomy scar that healed spontaneously. One patient (0.8%) developed recurrent ulcer-in-ano complete with a sentinel pile and a hypertrophied papilla 2 years after the original operation and

underwent a second fissurectomy with superficial midline sphincterotomy. None of the patients developed incontinence, anal strictures, or keyhole deformity. Except 3 (2.5%) who had recurrences of their fissures, the remaining 118 (97.5%) were satisfied with the result.

Gingold (1987) described sphincterotomy with partial fissurectomy as an outpatient procedure under bupivicaine local anaesthesia and reported a series of 86 patients with a median follow-up of 2 years. Three patients had recurrence, giving a success rate of 96.4 %, with no complications or disturbed continence.

Di Castro *et al.* (1997) reported on 195 patients who underwent fissurectomy with posterior midline sphincterotomy with anoplasty (FPSA). The fissure was excised with an elliptical incision until healthy tissue was exposed, and a sphincterotomy was made in the posterior midline. The cut edge of the rectal mucosa was then drawn downwards and sutured to the lower margin of the IAS. The wound healed satisfactorily in all patients after a mean time of 4 weeks.

Recent therapeutic agents in the conservative treatment of fissures have complemented surgical treatment. Engel *et al.* (2002) used topical isosorbide dinitrate after fissurectomy to enhance postoperative tissue perfusion and promote wound healing. In 17 patients with fissures not responding to conservative management fissurectomy was performed. There were no postoperative complications. All wounds had healed by 10 weeks and no fissure recurrence was seen after a median follow-up of 29 months.

1.5.4. ANAL ADVANCEMENT FLAPS

Whilst the classic high-pressure fissure responds well to lateral internal sphincterotomy, the anal island advancement flap is advocated for patients with primary or recurrent fissures and for women with a complicated obstetric history with low resting anal canal pressure. This particular group of patients do not benefit from sphincterotomy as this is more likely to precipitate incontinence. In order to aid selection of suitable cases, manometric and endosonographic evaluation is often employed. This operation avoids further disruption to the internal sphincter, and avoids factors that might otherwise jeopardise continence. Skin flaps can either be triangular (Y-V), or a square-shaped sliding graft. Oh and Zinberg (1982) advocated the use of C-anoplasty as it extended the pedicle without compromising the vascular supply.

Samson and Stewart (1970) reported a Y-V anoplasty performed in 2072 patients from 1964 to 1968. The infected fissure with crypt bearing haemorrhoidal tissue was excised and the defect closed with a broad based split skin graft. Though the authors did not state their success rate they reported few complications: 10 fissures recurred, and 7 patients had mild stenosis.

Nyam *et al.* (1995) performed an island advancement flap in 21 patients with anal fissures who had weak sphincters. The preoperative median resting anal pressure was 66 (range 43-90) cmH_2O, these values were significantly lower than controls. Endoanal

ultrasonography demonstrated sphincter defects in 15 of these patients. All fissures healed and, at a median follow up of 18 (range 2-28) months continence was maintained.

Leong and Seow-Chen (1995) prospectively evaluated 40 patients who were randomised equally to either receive a lateral sphincterotomy or rhomboid anal advancement flap. They showed that whilst all those in the former group healed, three in the latter group did not. No anal incontinence was recorded in any patient from either group. Of the sphincterotomy group, three were dissatisfied as they developed skin tags or temporarily painful scars at the operation site. In the anal advancement group, three were not satisfied as the wounds failed to heal and two underwent subsequent lateral sphincterotomy. The follow up did not include anal manometry.

1.6. ENDOANAL ULTRASOUND IMAGING OF THE ANAL SPHINCTERS

Endoanal ultrasonography has identified the effect of anal dilatation and lateral sphincterotomy on the anal sphincters. Speakman *et al.* (1991) examined the structure and function of the anal sphincters in 12 male patients who were incontinent following anal dilatation. The mean resting pressure was 49 cmH_2O, and the mean voluntary contraction pressure was 93 cmH_2O. In 11 of the 12 patients the IAS was disrupted with a mean loss of 153^{O} of the circumference. Anal dilatation resulted in fragmentation of the IAS, rather than an isolated defect. Three patients also had defects in the external anal sphincter and only one patient had a normal endosonographic appearance.

Nielsen *et al.* (1993) studied 32 patients with a median age of 48 (range, 19-78) years and a median follow up of 4 (range, 2-6) years after anal dilatation performed for chronic anal fissures. Four patients (12.5%) developed minor incontinence. Of 20 who accepted endosonographic follow-up, sphincter defects were found in 13 (65%); 2 of the 4 incontinent patients and 11 of the 18 continent patients had IAS defects. In 4 continent patients, the IAS was fragmented and had disrupted at more than one site. There was no description of associated external anal sphincter injury. Anal dilatation can be regarded as an uncontrolled tearing procedure that does damage the sphincter muscles with considerable detriment to anal function *(fig. 1.1.)*.

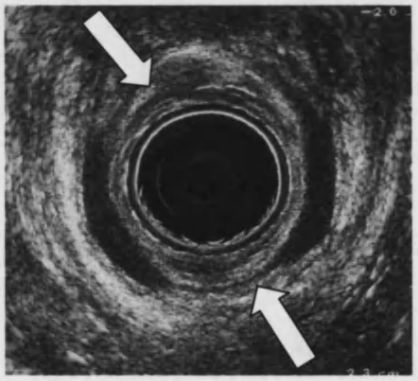


Figure 1.1. Endoanal ultrasound demonstrating the appearance of the internal anal sphincter (IAS) after manual dilatation for chronic anal fissure. The white arrows indicate the defects in the IAS.

Sultan *et al.* (1994) carried out a prospective evaluation of the extent of IAS division two months after open lateral sphincterotomy in 15 patients (mean age 39, range 26-67 years) with chronic anal fissure. The defect in the IAS corresponded to the site of surgical division, and was usually 20% of the circumference *(fig. 1.2).* The disruption in females was more extensive, as in 9 of 10 females the defect involved the whole length of the IAS, presumably related to the shorter anal canal in females. The authors recommended that as division of the IAS in most females was more extensive than intended, a limited division to the length of the fissure should be sufficient as Sultan *et al.* (1993) had identified occult external sphincter defects in 35% of primiparous and 44% of multiparous females following vaginal delivery.

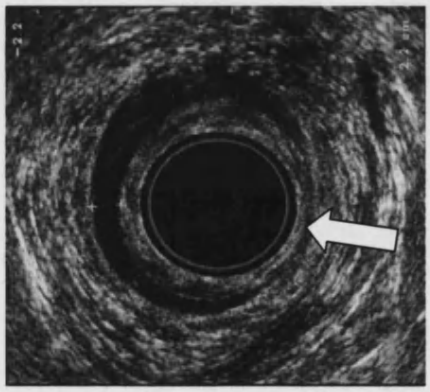


Figure 1.2. Endoanal ultrasound demonstrating the appearance of the internal anal sphincter (IAS) after lateral sphincterotomy for chronic anal fissure. The white arrow indicates the defect in the IAS.

Such is the current concern regarding sphincterotomy that Farouk *et al.* (1998) advocated perioperative endoanal ultrasound scan for patients with anal fissures to identify those at high risk of incontinence in whom an anal advancement flap should be a safer option to a sphincterotomy. Although a sphincteric defect after dilatation does not necessarily cause disturbance of continence it may manifest clinically later in time as a consequence of idiopathic degeneration [Vaizey *et al.*, 1997] or of childbirth [Burnett *et al.*, 1991].

It has become evident from these observations that patients, particularly females, should be appropriately selected and any form of treatment that disrupts the sphincter mechanism should be considered carefully. Endoanal ultrasonography has also identified inadvertent division of the EAS or inadequate division as reasons for failure of a fissure to heal [Farouk *et al.*, 1997; Garcia-Granero *et al.*, 1998].

1.7. CHEMICAL SPHINCTEROTOMY

This term refers to pharmacological manipulation of anal sphincter tone as an alternative modality to surgery for the treatment of anal fissures. Lateral sphincterotomy diminishes IAS hypertonia and reduces anal canal pressure; this in turn improves anal mucosal blood low and promotes healing of anal fissures. However, sphincterotomy can be associated with long-term disturbances of sphincter function. The optimal treatment for anal fissures is to induce a temporary reduction of anal canal pressure to promote healing of the fissure without permanently disrupting normal sphincter function. Broader

understanding of the intrinsic mechanisms controlling smooth muscle contraction has allowed pharmacological manipulation of anal sphincter tone [Lund and Scholefield, 1996; Madoff, 1998]. A reduction in anal sphincter tone is achievable by enhancing IAS relaxation through direct action on internal anal sphincter smooth muscle cells. These mechanisms serve to reduce intracellular Ca^{2+}, which reduces the tonic state of the muscle. These can occur through nitric oxide donation, direct intracellular Ca^{2+} depletion, muscarinic receptor stimulation, α-adrenergic inhibition, or β-adrenergic stimulation [Cook *et al.*, 2001]. Manipulation of these mechanisms does not effect the contraction of the striated external anal sphincter whose contractile mechanisms function at the level of the neuromuscular junction.

1.7.1. NITRIC OXIDE DONORS

O'Kelly *et al.* (1993) recognised nitric oxide as the predominant neurotransmitter mediating neurogenic relaxation of the human IAS. They also demonstrated that sodium nitroprusside, an exogenous source of nitric oxide, mimicked the effect of electrical field stimulation in relaxing the human IAS *in vitro*. Glyceryl trinitrate (GTN) and isosorbide dinitrate have also been shown to act as nitric oxide donors, and are available as ointments for topical administration. Nitric oxide donors probably aid healing through an increase in local blood flow secondary to a reduction in intra-anal pressure and perhaps also by vasodilatation of the vessels supplying the anal musculature. Kua *et al.* (2001) showed that application of 0.2% GTN ointment onto the anoderm increased the mean

blood flow over the anal fissure region. This effect lasted throughout a one hour study period.

The optimal therapeutic dose

Loder *et al.* (1994) investigated the feasibility of "reversible chemical sphincterotomy" by local application of GTN. Their study was conducted with 3 phases. In an initial investigation of two patients to establish the optimal drug concentration aliquots of 0.2%, 0.5%, 1% and 2% were used. Headaches lasting several hours were reported in both patients with the 0.5%, 1% and 2% doses and in only one patient lasting 30 minutes with the 0.2% formulation. The second phase investigated the effect of 0.2% GTN in 10 subjects, with a mean age of 39 years, two of whom were without anorectal conditions, two with pruritis ani, four with haemorrhoids, one with a fissure and the remaining one with anal pain. 0.2% GTN caused the maximum resting anal canal pressure to significantly decrease by 27% from a mean of 99 cmH_2O to 73 cmH_2O. In a randomised trial 20 patients treated with 0.2% GTN the maximum resting anal canal pressure decreased significantly from a mean of 103 cmH_2O to 79 cmH_2O, while in those treated with placebo the resting pressure change from a mean of 95 cmH_2O to 88 cmH_2O was not significant.

Gorfine (1995a) treated 15 patients, 11 who had acute posterior midline fissures, one with an acute anterior fissure, and three who had posterior midline ulcers, with 0.5-1g of 0.5%

GTN ointment used four times daily. All patients reported relief of anal pain within 3 to 4 minutes after application. Of the 12 patients with superficial anal fissures, 10 (83%) were healed within two weeks and the remaining 2 healed after four weeks of treatment. Of the patients with chronic anal ulcers though one had symptomatically improved after two weeks, requested sphincterotomy and healed. The remaining 2 patients had also improved symptomatically but failed to heal after two months. They refused sphincterotomy. Mild transient headaches were reported in 5 (33%) patients. Gorfine (1995b) used 0.3% GTN four times daily in 40 patients with anal fissures and 14 with anal ulcers. Compliance with therapy occurred in 30 and 11 patients respectively. In those with anal fissures 9 (30%) within two weeks, in 21 (70%) within four weeks or less and in 23 (77%) within eight weeks or less. In those with anal ulcers 2 (18%) healed within two weeks, 3 (27%) within four weeks or less and in 6 (54%) within eight weeks or less. Headaches occurred in 12 (22%) of 54 patients.

Watson *et al.* (1996) applied GTN in increasing concentrations from 0.2%-0.8% to the anal margin in 19 patients with anal fissures. If, after application of a dose of GTN, the anal canal pressure failed to decrease by 25% without side effects, the next higher concentration was used. Of 13 that attended follow up at 6 weeks the fissure healed in 9 (69%), one healed with 0.2%, five with 0.3%, one with 0.4% and two required 0.8%. Five patients demonstrated tachyphylaxis to GTN.

Lund *et al.* (1996) found that while a dose of 0.4% in two subjects caused headaches whereas 0.2% caused no side effects in 4 volunteers. They then treated 21 patients

suffering from chronic anal fissures with 0.2% GTN twice daily for 4-6 weeks. The mean ± s.d. maximum resting anal canal pressure decreased significantly from 118.7 ± 45.0 cmH_2O to 70.3 ± 34.1 cmH_2O over 20 minutes after application. Healing occurred in 11 (52%) at 4 weeks and in 18 (86%) at 6 weeks. Lund and Scholefield (1997a) reaffirmed in a further study of 39 consecutive patients with chronic anal fissures, seven of whom had recurrences after previous surgery, the effectiveness of treatment with 0.2% GTN. The maximum anal canal pressure decreased significantly from 122.1 ± 44 to 72.5 ± 33.3 cmH_2O 20 minutes after application. Healing occurred in 14 (36%) at four weeks and in 33 (85%) at six weeks. All 7 patients with recurrent fissures healed with GTN ointment. Mild headaches occurred in 8 (24%) and 5 fissures that had healed at four weeks recurred, of these 4 were again successfully treated with GTN.

Carapeti *et al.* (1999a) investigated the optimal therapeutic dose with the least side effects and long-term benefit. In a double blind controlled trial, patients were randomised to receive placebo, 0.2% GTN, or increasing concentrations of GTN starting with 0.2% for 8 weeks. After 10 weeks (which included 8 weeks of treatment) healing was observed in 7 (32%) of 22 patients on placebo, 15 (65%) of 23 on 0.2% GTN, and 16 (70%) of 23 patients on escalating doses of GTN. Nine (39%) of 23 fissures had healed after six weeks with high dose of GTN compared with 5 (22%) of 23 on 0.2% GTN. Headaches were reported by 33 (72%) of 46 patients on GTN compared with 6 (27%) of 22 on placebo. All groups described a significant reduction in mean pain scores after 2 weeks of therapy. There was a reduction in the maximum resting anal canal pressure from pre-treatment baseline to 8 weeks and this was more pronounced after GTN, with a mean

reduction of 32 ± 15 cmH_2O as compared with placebo which resulted in a decrease of 19 ± 19 cmH_2O. The median follow up was 9 (range 6-14) months during which recurrences were observed in 3 (43%) of 7 treated with placebo, 5 (33%) of 15 treated with 0.2% GTN and 4 (25%) of 16 treated with higher doses of GTN. This study affirmed that there was no advantage in using doses of GTN higher than 0.2%, as although higher doses healed the fissures more quickly, this did not prevent relapse. They attributed the high healing rate of placebo to the fact that many patients with chronic fissures could heal spontaneously.

Palazzo *et al.* (2000) re-explored the use of 0.5% GTN in 45 patients. At 6 weeks, 73% of the fissures healed and at 3 months there were no recurrences; 84% had headaches, and 11% discontinued treatment because of that. This study affirmed that high dose of GTN healed fissures, but with higher incidence of side effects. Ward *et al.* (2000) using 0.5% GTN healed 12 (75%) of 16 fissures but 9 (56%) of the 16 had headaches. Ciccaglione *et al.* (2000) have also shown that higher doses (2% as compared with 0.2%) of GTN are of no added benefit in the healing rate or the reduction in maximum resting anal canal pressure.

Scholefield *et al.* (2003) conducted a double blind, multicentre, randomised controlled trial dose finding study with 0.1%, 0.2%, 0.4% GTN and placebo. Initial analysis showed that, after 8 weeks of treatment, the healing rate in the placebo group was 37.5% compared with 46.9% for 0.1%, 40.4% for 0.2% and 54.1% for 0.4% GTN. None was significantly better than the placebo response. After excluding fissures without secondary

criteria for chronicity, the healing rate in the placebo group was 24%, compared with 50% for 0.1%, 36% for 0.2%, 57% for 0.4% GTN. These values were statistically significantly different for the placebo group compared with the 0.1% and 0.4% GTN groups. This apparent effect of GTN on the chronic anal fissures but not the acute fissures led the authors to conclude that perhaps the definition of chronicity related to fissures may need to be reviewed.

These few reports indicate that while GTN has a therapeutic role headaches constitute a major disadvantage in its use and patients' compliance outside clinical trials is not sufficiently strict.

Comparative studies on the effectiveness of GTN

Lund and Scholefield (1997b) in the first randomised trial treated 80 consecutive patients with topical application of either 0.2% GTN or placebo twice daily. After 8 weeks 26 (68%) of 38 treated with GTN healed as compared to 3 (8%) of 39 treated with placebo. Two patients from the fissure group were excluded because fissures were not identified at the time of inclusion. One patient from the placebo arm failed to attend. The maximum resting anal canal pressure fell significantly from 115.9 ± 31.6 to 75.9 ± 30.1 cmH_2O after application of GTN compared to 118.1 ± 44.6 to 111.5 ± 42.0 cm H_2O after placebo. The anodermal blood flow, measured by laser flowmetry, significantly increased from 32.4 ± 27.5 to 42.8 ± 27.6 units after application of GTN but with placebo the change from 24.9 ± 11.1 to 28.1 ± 10.2 units was not significant.

Bacher *et al.* (1997) randomised 12 acute fissures, and 8 chronic fissures to GTN and 10 acute and 5 chronic fissures to topical local anaesthetic. At 2 weeks GTN healed 60% of the fissures, 11 (92%) of 12 acute fissures and 1 (13%) of 8 chronic fissures; while none treated with local anaesthetic gel healed. At 4 weeks GTN healed 80% of cases, 11 (92%) of 12 acute fissures and 5 (63%) of the 8 chronic fissures; while 40% healed with local anaesthetic gel, 5 (50%) of 10 acute fissures and 1 (20%) of 5 chronic fissures. Those who had healed by 2 weeks with GTN showed a significant reduction in maximum anal canal pressure from 110 (78-156) to 87 (60-120) cmH_2O but in those that healed by 4 weeks there was no significant decrease in maximum anal canal pressure. GTN caused headaches in 20% of patients.

Kennedy *et al.* (1999) randomised 43 patients with chronic anal fissures, 24 for GTN and 19 for placebo treatment thrice daily for three weeks. After four weeks if the fissures remained unhealed in the GTN group, treatment was continued until healing was achieved or lateral sphincterotomy was performed if symptoms were severe. At one week in the GTN group the maximum resting anal canal pressure reduced significantly from 125 ± 8 cmH_2O to 107 ± 7 cmH_2O and had increased to 119 ± 8 cm H_2O by 4 weeks. The reduction in anal canal pressure in the placebo group at one week (117 ± 4 cmH_2O) and 4 weeks (113 ± 5 cmH_2O) was not significant. At four weeks eleven had healed with GTN and three with placebo.

GTN in clinical practice

Whilst GTN is advocated as first line treatment for chronic anal fissures with encouraging results, concerns about its effectiveness in clinical practice outside clinical trials emerged in other reports. Pitt *et al.* (1999) published a report of a consecutive series of 45 patients with chronic anal fissures with a median duration of 6 months treated with 0.2% GTN and followed up for a median of 6 (1-11) months. Patients were reviewed every 4 weeks and GTN treatment was continued if they were improving, otherwise they were advised to have internal sphincterotomy if the fissure did not heal after 12 weeks. Twenty-six (58%) developed headaches during treatment and 5 abandoned treatment in favour of surgery. Twenty-two (49%) healed, but 10 recurred and so only 12 (27%) of the original 45 remained healed during the follow up period. The median time to healing of the fissure was 6 (range 2-12) weeks; recurrences started 7 weeks after stopping treatment. The authors concluded that GTN was a useful adjunct to therapy but internal anal sphincterotomy remained the mainstay of treatment.

Dorfman *et al.* (1999) conducted a retrospective review of 31 patients with chronic anal fissures who were managed by twice-daily 0.2% GTN. Of 27 respondents 18 (67%) had complied with treatment and, of 15 (56%) who had complete symptomatic relief after 3 to 10 weeks of therapy, 4 (27%) recurred after a median follow up of 6 months and 21 (78%) of the 27 had headaches or light headedness, sufficiently severe for 2 (10%) patients to cease therapy.

Jonas *et al.* (1999) evaluated the results of topical treatment with 0.2% GTN for 6 weeks in 49 consecutive patients; the fissures healed in 21 (43%), in 2 the fissures healed spontaneously after the patients discontinued their medication, and in 26 (53%) patients with persistent symptoms after completing treatment, the fissure healed after patients underwent a lateral internal sphincterotomy. The investigators concluded that despite increasing concerns about surgery it still had a valuable role in healing of chronic anal fissures.

Altomare *et al.* (2000) conducted a multicentre, randomised, placebo-controlled, double blind trial where 132 consecutive patients received either 0.2% GTN or placebo twice daily for 4 weeks. One hundred and nineteen patients completed the trial and the two groups were matched for gender, age, duration of symptoms, site of fissure, and duration of treatment. 29 (49.2%) of 59 healed after GTN and 31 (51.7%) of 60 healed after placebo. The mean pain score after one week of GTN treatment decreased significantly from 7.56 ± 1.8 to 4.13 ± 2.7 and there was also a significant reduction from 6.91 ± 2.3 to 3.97 ± 2.8 after one week of placebo. Anal manometry was available in 93 patients. The maximum resting anal canal pressure significantly fell from 97.3 ± 16 to 83.2 ± 16 mmHg in the GTN group and from 92.1 ± 14 to 82.4 ± 17 mmHg in the placebo group. This trial reaffirmed clinical doubt of the superiority of 0.2% GTN over placebo.

Hasegawa *et al.* (2000) followed up 16 patients with acute fissures and 40 patients with chronic anal fissures after treatment with 0.2% GTN. Ten (63%) of 16 acute fissures healed at 4 weeks and 13 (81%) by 8 weeks, whilst of the chronic fissures 13 (33%)

healed by 8 weeks and 20 (50%) by 12 weeks. Headaches were seen in 8 (14%), 7 discontinued treatment; and 26 (46%) experienced mild headaches. Five (22%) patients had recurrences, 4 were treated successfully with a further course of GTN and the other patient required a lateral sphincterotomy, and healed.

GTN vs lateral sphincterotomy

Oettlé (1997) randomised 24 patients to either GTN or sphincterotomy. All fissures healed by 4 weeks after sphincterotomy and, at a median follow up period of 22 (8-34) months, there were no recurrences and no patient developed incontinence. Of those 12 patients treated with GTN, in 10 (83%) the fissures healed by 4 weeks and in the other 2 patients the fissures failed to heal after two more weeks of treatment until they underwent sphincterotomy. At follow up, those fissures that had healed with GTN remained healed.

Two recent randomised controlled trials concluded that GTN was not superior to lateral sphincterotomy as treatment for chronic anal fissures. Richard *et al.* (2000) presented a randomised study by the Canadian Colorectal Surgical Trials Group in which the fissure healed at 6 weeks in 34 (89.5%) of 38 patients in the sphincterotomy group, compared with 13 (29.5%) of 44 treated with GTN, of whom 5 (38.4%) of 13 relapsed. The respective healing rates at 6 months were 35 (92.1%) and 12 (27.2%); 20 (45.4%) treated with GTN required a sphincterotomy and 8 (23.2%) patients discontinued treatment because of a headaches. The group concluded that internal sphincterotomy was superior to GTN and would remain the treatment of choice for the treatment of chronic anal

fissures. Evans *et al.* (2001) reported healing in 26 (97%) of 27 patients treated by sphincterotomy compared with 20 (60.6%) of 33 patients treated with GTN for 8 weeks; 12 patients who failed GTN treatment underwent sphincterotomy.

Libertiny *et al.* (2002) conducted randomised trial of 0.2% GTN and lateral internal sphincterotomy was conducted in 70 patients were randomised, with 35 in each group. Of those administered GTN, 19 (54%) of 35 healed and the remaining 16 were treated by sphincterotomy. Recurrences by six months were observed in three patients initially treated successfully with GTN, the other 16 that had initially healed had still healed by 24 months. All those that were operated on had healed, but one patient's fissure recurred after eight months.

The characteristics of the anal fissure and healing

Pitt *et al.* (2001a), concerned about the poor effectiveness of GTN, investigated 42 males and 22 females, mean age 37.5 years, with chronic anal fissures of a median duration of 7 (range 3-180) months. The fissures were located anteriorly in 17 (27 %) and 19 (30 %) were associated with sentinel piles. Patients were treated with 0.2% GTN topically twice daily and reviewed 4 weekly, and remained on GTN if there was symptomatic relief, otherwise they were offered sphincterotomy. Sphincterotomy was also offered if after 12 weeks treatment with GTN the fissure did not heal. The follow up period extended to 32 weeks. Initially 26 (40.6%) healed, but 12 (46.2%) had a recurrence within 32 weeks, therefore 14 (21.9%) remained healed in the long term. By completion of the study 20

patients underwent lateral internal sphincterotomy; the remaining patients who had not healed with GTN declined surgery. Headaches were observed in 41 (64 %); of these 10 (15.6%) stopped therapy. According to a Cox model multivariate analysis of sex, age, duration and site of the fissure, presence of sentinel pile, presence of childbirth and constipation factors related to failed treatment were the duration of greater than 6 months and the presence of a sentinel pile. The authors suggested that these patients should be warned that topical GTN therapy was more likely to fail, and earlier sphincterotomy was advised.

Long term results of GTN treatment

Recent published reports have reaffirmed the poor long-term results in the treatment of chronic anal fissures. Lund and Scholefield (1998) then assessed long-term follow-up of patients with chronic anal fissures treated with GTN. 41 patients who had previously healed with GTN returned a questionnaire with a median follow up 28 (range 24-38) months. 30 had no further symptoms and 11 developed a symptomatic relapse. Of these latter patients, 3 underwent sphincterotomy, 6 healed with further GTN and 2 settled spontaneously.

Kennedy (1999) conducted a randomised, double-blind trial, placebo-controlled trial to test the effectiveness of GTN and showed that both groups had a significant reduction in pain scores by 4 weeks. Long-term follow-up was conducted in 17 patients with a mean time of 28.5 (range 26-31) months. Of 8 patients who had initially healed with GTN, 5

recurred, a further course of GTN in these patients healed 2; 2 underwent sphincterotomy and 1 remained unhealed. Within the group of 17 a total of 10 (59%) had healed with GTN and 5 (29%) had undergone sphincterotomy.

Graziano *et al.* (2001) compared 0.25% GTN treatment in 22 patients (16 chronic and 6 acute fissures) with 21 (16 chronic and 5 acute fissures) who had received placebo treatment. The mean follow up was 39 weeks. The healing rate with GTN was 75% for chronic and 83% for acute fissures, and the recurrence rate for the chronic fissures at 9 months was 67%. Only 1 (6%) of 16 of the chronic fissures healed with placebo. Whilst GTN was effective for both acute and chronic fissures, there was an appreciable rate of recurrence.

Systemic levels of GTN

Jonas *et al.* (2001a) investigated whether systemic levels of GTN following topical application correlated with the measured reduction in anal pressure. 30 healthy volunteers underwent static anal manometry for 10 minutes before and 2 hours after application of 0.2% GTN to the anoderm. Blood samples were taken regularly for 3 hours post-application. The MRP was significantly reduced after 10 minutes but had returned to pre-treatment values by 3 hours. Whilst the pulse was statistically unchanged during the study, the systolic blood pressure was significantly lower 20-90 minutes after application, and diastolic pressure was decreased throughout the study. Headaches were experienced by 14 of 30 volunteers after a median (range) of 41 (4-120) minutes,

persisting for 74 (30-176) minutes, with an intensity score of 19 (5-30) mm represented on a 100-mm visual analogue scale. There was no correlation between plasma GTN concentration, MRP, and the onset, duration or intensity of headaches.

Isosorbide Dinitrate

Isosorbide dinitrate (ISDN) is an alternative nitric oxide donor that has been used successfully in the treatment of anal fissures. Schouten *et al.* (1996c) treated 34 patients with 1% ISDN ointment applied 3 hourly to the anoderm. All patients reported mild transient headaches during the first 2 days that ceased by 10 days, but no patient stopped treatment. At 6, 9 and 12 weeks the fissures healed in 14 (41%), 22 (65%) and 30 (88%) patients respectively. The mean maximum resting anal pressure was reduced from 111 mmHg to 86 mmHg at 3 weeks and to 96 mmHg at 6 weeks. The mean duration of follow up was 11 months and during this period in 2 (7%) the fissure recurred. Of the four patients that had a persistent fissure after 12 weeks of treatment, two were treated by lateral sphincterotomy and two by fissurectomy and anoplasty.

Lysy *et al.* (1998) used Isoket® spray, isosorbide dinitrate, to treat 41 patients with chronic anal fissure in a dose of 1.25mg or 2.5mg thrice daily for 4 weeks. Of 41 patients 34 (83%) healed within 4 weeks. The average time for symptoms to resolve was 6.5 ± 0.7 days. In 6 (15%) the fissures did not heal after a further 4 weeks of treatment; they underwent lateral sphincterotomy. One patient discontinued treatment because of headaches, and overall seven patients had mild headaches during treatment. Six of the 34

that had initially healed suffered recurrence, but responded to a further 4 weeks of treatment. In patients whose treatment was successful, the reduction in mean resting pressure was 32.5% (from 110.6 ± 2 to 70.6 ± 1.3 mmHg), whereas the decrease in those that did not respond was 16% (105 ± 2 to 90 ± 2.6 mmHg).

Were *et al.* (2001) randomised 20 patients to ISDN and 17 to a placebo. Both groups were treated for a median duration of 5 (1-10) weeks. In the ISDN group the fissures healed in 17 (85%) compared with 6 (35%) controls with two recurrences in each group. Headaches occurred in 9 (45%) treated with ISDN and in 3 (18%) who had a placebo.

Engel *et al.* (2002) hypothesised that chronic anal fissures unresponsive to conservative treatment was due to the fact that the fissure was an unstable scar tissue. The investigators selected 17 patients whose mean age was 46 (29-78) years with painful fissures who had failed to heal with 1% ISDN. These patients were treated by fissurectomy followed by topical ISDN. The aim was to create a fresh surgical wound and to promote its healing by improving tissue perfusion with a nitric oxide donor. At six weeks healing was complete in all except one who required treatment until 10 weeks. At a median follow-up of 29 (23-36) months there were no recurrences and none complained of incontinence to solid, liquid faeces, or gas.

1.7.2. CALCIUM ANTAGONISTS

Nifedipine is a dihydropyridine calcium-channel blocker; less correctly referred a 'calcium-antagonist,' which inhibits calcium ion entry through voltage-sensitive areas of vascular smooth muscle and myocardium. It reduces myocardial contractility, depresses the formation and propagation of electrical impulses within the heart, and decreases coronary and systemic vascular tone. Nifedipine is used for the treatment of angina and hypertension. Its side effects are associated with vasodilatation and include flushing and headache (which become less obtrusive after a few days), and ankle swelling (which may respond only partially to diuretics).

Chrysos *et al.* (1996) conducted the first clinical study of the role of calcium antagonists on anal canal pressure based on the rationale that nifedipine, a calcium channel antagonist, decreased lower oesophageal sphincter pressure in achalasia. Ten patients with non-prolapsing haemorrhoids and/or anal fissures, mean (s.d.) age 52 ± 11 years; and 10 controls, mean (s.d.) age 45 ± 9 years underwent anal manometry before and 30 minutes after sublingual administration of 20mg nifedipine. The maximum resting anal canal pressure decreased significantly in both groups, in patients from 119.5 ± 15.7 cmH_2O (mean, s.d.) to 82.4 ± 12.9 cmH_2O, and in controls from 87.5 ± 8.4 cmH_2O to 66.8 ± 11.9 cmH_2O, a reduction of resting pressure noted for patients and controls was 32% and 24% respectively. The mean maximum squeeze pressure in both groups was unchanged. This was as expected as the external anal sphincter is striated muscle and as such is unaffected by nifedipine. The investigators noted that nifedipine also influenced the frequency and amplitude of slow waves in both groups; the presence, frequency and amplitude of ultra-slow waves in patients, but not in controls; and the frequency of anal

sampling events in the patient group. These results indicated that nifedipine also had an inhibitory effect on the phasic activity of the IAS. Nifedipine did not influence the blood pressure and heart rate significantly. None reported any dizziness, fatigue or nausea, but 2 controls and one patient reported transient headache or showed flashing of the face that settled within 30 minutes.

Antropoli *et al.* (1999) in a randomised, double-blind, multicentre trial, investigated the efficacy of local application of 0.2% nifedipine gel twice daily for 3 weeks in healing acute fissures in 141 patients compared to a control group of 142 patients treated with 1% lidocaine and 1% hydrocortisone gel. At three weeks 134 (95%) healed with nifedipine compared to 71 (50%) in the control group. The maximum resting anal canal pressure decreased significantly by 30% in the nifedipine group from a mean of 72.5 ± 10.1 mmHg to 50.5 ± 10 mmHg after three weeks, and the squeeze pressures also reduced significantly by 16.8 % from 130.5 ± 19.2 mmHg to 108.5 ± 18.6 mmHg. In the control group the reductions in resting pressure (from 70.5 ± 9.1 mmHg to 63.5 ± 9.0 mmHg) and squeeze pressure (from 129.5 ± 20.1 to 119.5 ± 17.3 mmHg) were not significant. No patient treated with nifedipine showed any systemic side effect, probably due to lower systemic absorption than that seen with sublingual nifedipine.

Cook *et al.* (1999a) evaluated oral nifedipine on resting anal pressure and on fissure healing. The authors felt that an oral preparation would overcome some of the confusion that existed with the site of application and dose of topical preparations, and that it might improve patient compliance. Eight volunteers of median age 31 (range 20 to 35) years

and 15 patients of median age 34 (range 20 to 60) years with chronic anal fissures were treated with 20mg nifedipine orally twice daily. In the volunteers, the mean maximum resting anal canal pressure fell significantly by 35% from 85 to 54 cmH_2O after the initial dose and after five days by 28% to 62 cmH_2O. There was no significant change in the squeeze pressure before (122 cmH_2O) or after the initial dose (121 cmH_2O) or at 5 days (128 cmH_2O). The diastolic blood pressure did not change significantly. In the 15 patients the initial dose of nifedipine caused a 36% significant fall in the maximum resting anal canal pressure from 102 to 65 cmH_2O. There was no change in the maximum squeeze pressure (from 93 vs. 95 cmH_2O). The diastolic blood pressure fell significantly after the first dose, but this was not sustained. Five patients developed headaches, 10 noted flushing and one developed ankle oedema. No patients developed postural hypertension or incontinence. In three (20%) patients the fissures healed by six weeks and in nine (60%) after eight weeks. Another three patients were asymptomatic and declined further treatment, one patient healed with topical GTN and one underwent a sphincterotomy, one patient recurred within the first month, but responded to topical GTN treatment. Cook and Mortensen (2000) commented on the reduction of squeeze pressure reported by Antropoli *et al.* (1999) that has not been reported in other studies and were unable to offer an explanation.

Diltiazem, a non-dihydropyridine calcium-channel blocker, also effects vascular smooth muscle relaxation and dilatation. Its clinical use is similar to nifedipine but it has cardiac-depressant activity, slowing automaticity and conduction through the atrioventricular node but it induces less vasodilatation.

Carapeti *et al.* (1998a, 1998b, 1999b) demonstrated that a single dose of oral diltiazem significantly lowered the mean resting anal sphincter pressure by 21%, from 97 ± 17 to 77 ± 11 cmH_2O and in a twice-daily dose by 17%, from 97 ± 17 to 80 ± 14 cmH_2O; two (20%) of 10 subjects described postural dizziness. The investigators sought to achieve a similar effect with a topical dose of diltiazem gel. A maximal effect was produced by 2% diltiazem. The mean resting anal pressure was reduced by 28%, from 105 ± 12 to 76 ± 11 cmH_2O. This effect lasted three to five hours after application, and no side effects were observed in the 10 subjects that were studied. Higher concentrations of 5% and 10% topical diltiazem produced no additional effect. It was noted that neither the oral nor the topical preparations of diltiazem caused an increase in the anodermal blood flow measured using a laser doppler flowmeter. These encouraging reports offered an alternative to GTN in the treatment of chronic anal fissures.

Carapeti *et al.* (2000) proceeded to examine the effectiveness of 2% topical diltiazem gel applied thrice daily for eight weeks in 15 patients with chronic anal fissures whose median age was 37 (range 26 to 66) years. The starting resting anal pressure was elevated in 14 patients (>120 cmH_2O); one patient had a normal resting pressure of 85 cmH_2O. At eight weeks 10 (67%) of 15 the fissures had healed. Of the remaining five, one was asymptomatic and had a normal resting pressure, despite persistence of the fissure, and four remained symptomatic and opted for internal anal sphincterotomy. There was no significant difference in the pre-treatment maximum resting anal canal pressure between responders (median 120; range, 85-156 cmH_2O) and nonresponders (median 136; range,

110-140 cmH_2O) or in the post-treatment maximum resting anal pressure between responders (median 88; range, 80-104 cmH_2O) and nonresponders (median 94; range, 82-142 cmH_2O). No headaches or side effects were reported. In the whole group there was an overall significant reduction in mean maximum resting anal canal pressure of 24.4% and median pain scores after treatment. This study also demonstrated that there was no significant difference in anodermal blood flow before and during treatment measured by laser doppler flowmetry.

Knight *et al.* (2001) reported on a prospective assessment of 71 patients, median age 39 (range 12-76) years with chronic anal fissures, treated with 2% topical diltiazem gel twice daily. At the first review, after an eight week course, 47 (73%) of 64 who attended the first review healed, 17 with an unhealed fissure were given the option of a further course of diltiazem, topical 0.2% GTN or internal sphincterotomy. Of these, 12 had a second course of diltiazem, of whom 8 healed after a further course of treatment. At median follow up of 32 (range 14-67) weeks 27 (66%) of 41 assessed remained asymptomatic, seven had mild symptoms with healed fissures and seven had a fissure recurrence of whom three healed with further topical diltiazem gel, three healed with topical GTN and one underwent internal sphincterotomy.

Jonas *et al.* (2001b) investigated the role of oral diltiazem as a potential treatment for anal fissures and randomly assigned 24 patients, median age 35 (range 18-61) years, to receive oral (60mg) diltiazem and 26 patients, median age 35 (20-80) years to have topical 2% diltiazem twice daily for eight weeks. The maximum anal resting pressure fell

significantly after the first dose in both groups; by 15% from a mean of 95 to 81 cmH_2O on oral treatment and by 23% from a mean of 102 to 79 cmH_2O on topical treatment. At eight weeks the fissure healed in 9 (38%) who were on oral diltiazem and in 17 (65%) who had topical diltiazem. Oral diltiazem caused side effects in 8 patients; headaches in two, nausea or vomiting in three, a generalised rash in two, and a reduced sense of smell and taste in one patient. Seven of these patients did not complete the 8 weeks prescribed course of treatment. No patient on topical diltiazem reported side effects. There were no changes in pulse or blood pressure which may be due to the low oral dosage and to negligible systemic absorption of the topical preparation.

Kocher *et al.* (2002) randomly allocated 29 patients with fissures, median age of 39 (range 21-73) years, to receive 0.2% topical GTN twice daily and 31 patients, median age of 45 (21-73) years, to receive 2% topical diltiazem also twice daily. After 6-8 weeks, 21 (72%) in the GTN group experienced side effects (mainly headaches) compared with 13 (42%) in the diltiazem group. Three patients treated with diltiazem reported perianal itching or pruritis severe enough to discontinue treatment, but no patient reported incontinence. Symptomatic relief was achieved in 25 (86%) patients at 8 weeks in the GTN group, of these 12 healed and 13 improved. Similar relief was achieved in 24 (77%) patients in the diltiazem group, of whom eight healed and 16 improved. Thus, as the efficacy of topical diltiazem is similar to GTN with lesser side effects it was recommended as a first line for treatment for chronic anal fissures.

Jonas *et al.* (2002) have presented evidence that topical diltiazem healed GTN-resistant fissures. In 39 patients, median age 42 (range 20-80) years, with chronic anal fissures that had failed to heal on 0.2% GTN, 2% diltiazem gel was administered topically twice daily for 8 weeks. The 39 patients comprised 27 who had completed a course of GTN but had not healed. Of these, 12 (44%) healed with diltiazem and there were 12 who could not complete their GTN course due to headaches; of these 7 (58%) healed.

DasGupta *et al.* (2002) reported on 23 patients, median age of 45 (range 22-80) years who had symptoms for a median of 6 (range 2-36) months and were treated by 2% topical diltiazem as a thrice-daily application. The fissure healed in 11 (48%) of the group, usually by 8 weeks; the 12 remaining patients either underwent lateral sphincterotomy, used GTN or declined further intervention. This study reaffirmed the increasing acceptance for topical diltiazem and suggested using it to control symptoms while patients were waiting for internal sphincterotomy.

Griffin *et al.* (2002) contacted 35 of 50 patients, median follow-up period of 31 (range 27-37) months that had been treated by Jonas *et al.* (2001). Of those 17 responders, seven (41%) reported recurrent symptoms after a median duration of 6 (range 1 to 35) months with episodes lasting from 3 days to 2 weeks. Of these seven with symptomatic recurrence, four required further treatment with GTN, two of whom healed with GTN and two received injections of botulinum toxin and healed. None of the patients in the original responding group required surgery. The long term healing rate for GTN reported by Lund and Scholfield (1998) was 73% at a median follow up of 28 months and in

Kennedy's series (1999) 59% remained healed at a mean follow up of 28.5 months. These studies confirm that, whilst topical agents heal chronic fissures, there is an appreciable recurrence rate.

1.7.3. MUSCARINIC AGONISTS

Few studies have involved topical muscarinic agonists. Carapeti *et al.* (1999b) investigated 10 volunteers, median age 36 (range 21-49) years in whom 0.05%, 0.1%, and 1% bethanecol gel was applied to the anal verge. There was a dose-dependent reduction in the maximum resting anal canal pressure with a 24% significant reduction from a mean of 108 to 82 cmH_2O sustained for 4 (range 3-5) hours that was achieved with 0.1%; higher doses did not produce a greater effect. Carapeti *et al.* (2000) then went on to report the use of 0.1 % bethanecol gel in 15 patients with chronic anal fissures, median age 34 (range 20-75) years. After 8 weeks 10 (66%) were asymptomatic, in 9 (60%) the fissure had healed with relief of symptoms; in one asymptomatic patient the fissure persisted, 4 with unhealed fissures underwent sphincterotomy and the last patient withdrew because she was pregnant. There was a significant reduction in the maximum resting anal canal pressure, from a mean of 118 to 99 cmH_2O; and in the pain scores, from a median (range) of 5.5 (4.5-8) to 0.5 (0-3) after 8 weeks treatment. No headaches or side effects were reported. There was no significant change in the anodermal blood flow measured by laser doppler flowmetry before and during treatment.

1.7.4. SYMPATHETIC NEUROMODULATORS

Hieble and Ruffolo Jr. (1996) described the use of α-$_1$ adrenoceptor antagonists in the management of symptomatic urethral obstruction caused by α-adrenoceptor-mediated contraction of the stromal smooth muscle of benign prostatic hypertrophy. Eckardt *et al.* (1996) reported that inhaled salbutamol, a β_2–agonist, shortened attacks of severe pain in patients with proctalgia fugax.

Pitt *et al.* (2000) investigated the effect of indoramin, as an α-$_1$ adrenoceptor antagonist, on the internal anal sphincter in seven patients with chronic anal fissures, mean age 38 (range 24-48) years and six healthy volunteers, mean age 52.2 (range 30-71) years. Following a single 20 mg oral dose of indoramin, anal manometry was performed using a water-filled microballoon by a station pull-through method at one cm intervals, with equilibration of the pressure before a reading was taken. The measurements were repeated at one, two and three hours post ingestion. The mean maximum resting anal canal pressure reduced significantly from 106.9 to 68.6 cmH_2O, a 35.8% reduction in the patient group and from 84.2 to 52.2 cmH_2O, a 39.9% reduction in controls at one hour. These significant reductions were maintained at two and three hours in both groups. No adverse cardiovascular or other effects were experienced by any subjects in the study. The heart rate increased from a mean of 82.3 beats per minute to a maximum of 87.5 beats per minute after two hours and reduced to 84.7 after three hours. The mean blood pressure reduced significantly from 156.3 mmHg to 138.3 mmHg after 2 hours and

increased to 145.3 mmHg after 3 hours. The maximum resting anal canal pressure in both controls and patients with anal fissures was at 1 to 2 cm from the anal verge; this profile was not altered by indoramin. This initial study prompted further investigation into indoramin as a therapeutic agent for chronic anal fissures.

Ojo-Aromokudo *et al.* (1998) conducted a pilot study in volunteers and patients with chronic fissures using a dose of 4mg oral salbutamol. Combining Pitt *et al.'s* (2000) data with his results he noted that whilst there was no apparent difference in the α-adrenoceptors in the IAS of patients with anal fissures compared with controls, as measured by the reduction in anal canal pressure after the administration of indoramin, the β-adrenoceptors are probably up-regulated in patients with anal fissures, as the reduction in anal canal pressure was higher in patients with fissures than in controls. Regadas *et al.* (1993) have also demonstrated up-regulated β-adrenoceptors *in vitro* where supersensitivity to relaxation by isoproterenol, a β-adrenoceptor agonist, was demonstrated in IAS strips removed from patients undergoing lateral sphincterotomy, as compared with IAS strips removed from patients with third-degree haemorrhoids.

Pitt *et al.* (2001b) proceeded to conduct a double-blind randomised placebo-controlled trial of oral indoramin to treat 23 patients with chronic anal fissure. They treated 14, mean age 37 years, with 20mg oral indoramin; and nine, mean age 39 years with a placebo; seven patients in the indoramin group and two patients in the placebo group withdrew within the first 2 weeks of treatment due to side effects that included fatigue, dizziness, headache, dry mouth, nasal congestion, and retrograde ejaculation. At six

weeks the fissure healed in only one (7%) compared with two (22%) in the placebo group. The fissure that healed with indoramin recurred at three months. The investigators felt, on the basis of these results, that it was ethically inappropriate to continue with the study. Given that other similar agents that reduced resting anal canal pressure went on to heal chronic anal fissures it was not clear why indoramin had failed.

The conclusions of Ojo-Aromokudo *et al.* (1998) and Regadas *et al.* (1993) are in direct conflict with recent work by Acheson *et al.* (2002) who investigated 15 volunteers in a randomised, double-blind, placebo-controlled, cross-over trial of 4mg oral salbutamol. They showed that there were no significant differences between salbutamol and placebo when the fall in resting anal canal pressure at each time point were compared. Two hours after ingestion, the mean resting anal pressure was reduced by 3.0 % in the salbutamol group and 12.2 % in the placebo group and there were no significant changes in squeeze pressures. Blood pressure and heart rate all increased but did not reach statistical significance. Two volunteers developed a minor tremor and one had palpitations with the active drug. The investigators were doubtful whether sympathetic modulators could yield a therapeutic benefit for chronic anal fissures.

1.7.5. BOTULINUM TOXIN

Clostridium botulinum produces several toxins, of which types A, B and E have been linked to cases of botulism in humans. Botulinum toxin type A is of therapeutic value in various neurological and opthalmological disorders. Brooks in the 1950's suggested that botulinum neurotoxin type A might be used to reduce muscle hyperactivity [Schantz, 1994]. Botulinum neurotoxin type A (BoNT/A) was first used clinically by Scott (1980) as an injection into the extraocular muscles to treat strabismus.

Schiano *et al.* (1998) described the first use of botulinum toxin in the gastrointestinal tract in the treatment of achalasia. BoNT/A acts presynaptically by blocking acetylcholine release at the neuromuscular junction [Mandal and Robinson, 2001].

The first account of botulinum toxin in the treatment of an anorectal condition was for the treatment of 7 patients with anismus [Hallan *et al.*, 1988]. After injection of botulinum toxin into the striated muscle complex the symptom scores improved significantly and correlated with a significant reduction in the maximum voluntary and canal squeeze pressures. Joo *et al.* (1996) injected 6 units of botulinum toxin into either side of the paradoxically contracting muscle (at either the external anal sphincter (EAS) or the puborectalis muscle) in 4 patients with anismus that had failed treatment with biofeedback. All improved within 1 and 3 months after injection and 2 had sustained improvement for a period of 3 months to 1 year.

Jost (1997a) investigated the neurophysiological effect of Botox (botulinum toxin) on the EAS and demonstrated a temporary reduction of the amplitude of the optimum response after stimulation of the pudendal nerve without a change of nerve latency. This phenomenon via inhibition of acetylcholine release is translated clinically into incomplete contraction of the muscle. The mechanism of action on the IAS has only recently become apparent. Jones *et al.* (2002a, 2003) demonstrated that the contraction of isolated porcine IAS in an organ bath was inhibited by guanethidine consistent with this being mediated by sympathetic noradrenergic nerves. Although treatment with botulinum toxin increased myogenic tone by 38%, it reduced electrically field stimulated contraction. The investigators concluded that when botulinum toxin was used in the treatment of anal fissures the blockade of the sympathetic output probably outweighed its effect on increasing myogenic tone. Jones *et al.* (2002b) proposed that as the sympathetic drive on IAS in patients with chronic anal fissures was increased, botulinum toxin was a more specific pharmacological agent.

Jost and Schimrigk (1993) first described the use of botulinum toxin in the treatment of anal fissures. They injected 2.5 units of Botox (Allergan Pharmaceuticals, Irvine, Ca, USA) into the EAS on both sides of a chronic anal fissure in a 42-year old woman where attempts at conservative management had failed. Pain resolved after one day, the sphincter tone was reduced by the third day. The fissure healed and the sphincter tone returned to normal by 12 weeks. Jost and Schimrigk (1994) reported that 10 (83%) of 12 patients treated in the same manner healed by 3 months with no disturbance in continence or change in manometric measurements.

Gui *et al.* (1994) described the effect of three injections of 5 units of botulinum toxin into the internal sphincter in 10 patients. At one week pain disappeared in 7 patients and diminished in one. The resting pressure was reduced by 23.9%, whilst the voluntary squeeze pressure was unchanged. After 2 months the fissure healed in 7 (70%) patients, while the resting pressure remained low and the maximum voluntary pressure was unaffected.

Jost and Schimrigk (1995) reported their experience from 1992 in treating 26 patients with chronic anal fissures of average duration of 15 months. On the first day after injection in 19 (73%) the pain resolved and at 3 months 21 (81%) healed, with one case of mild incontinence that recovered completely. There were no recurrences in 19 patients assessed at six months and in 14 assessed at one year. Jost and Schimrigk collating their results in a pooled series of 50 patients with anal fissures, reported that, apart from temporary incontinence, an additional unwanted side effect of perianal thrombosis in 19.2% of 26 female patients treated.

Mason *et al.* (1996) demonstrated that either one or two injections of 0.125 ng or two injections of 0.5 ng of botulinum toxin into the lower fibres of the IAS in five male patients lowered the resting anal canal pressure without adversely affecting other anorectal physiological variables. The maximal resting anal canal pressure decreased from a mean (s.d.) of 146.6 (5.2) cm H_2O by 23.2 (5.6) cm H_2O. In two patients this was maintained for three months, in two patients the resting pressure was higher than pre-

treatment levels, while the voluntary squeeze pressure did not significantly change, and the last patient refused manometry.

Jost (1997b) injected either 2.5 or 5 units of botulinum toxin into the EAS in 100 patients with chronic anal fissures at one week 78% were pain-free. The fissures healed in 82% at three months and in 79% at six months, seven complained of incontinence for flatus for less than two weeks, and two female patients experienced faecal incontinence for one week. All with unhealed fissures had an internal sphincterotomy.

Espi *et al.* (1998) injected 20 patients with 5 units of Botox on either side of an anal fissure, and 16 patients with an additional 5 units below the fissure. The healing was 65% and 81% respectively at 6 months.

Maria *et al.* (1998a) in a double blind randomised trial compared 20 units of botulinum toxin injected into the internal anal sphincter with saline injections in 30 patients. The healing rates at 2 months were 73% in the treatment group compared with 13% in the control group. The reduction in resting anal canal pressure was 25%, in those injected with botulinum toxin, and was insignificant in the control group, while the voluntary pressure was unaltered in both groups. Maria *et al.* (1998b) investigated the effectiveness of larger doses of Botox injected into the IAS. The first group of patients with fissures received 15 units injected into the internal sphincter; the second group received higher doses of 20 and 25 units respectively. In a direct comparison it was found that whilst both 15 units and 20 units of Botox reduced resting pressure at one

month, only the higher dose caused in a sustained reduction at 2 months. Once again the maximum voluntary pressure was unchanged in both groups.

Brisinda *et al.* (1999) compared the effectiveness of 20 units of botulinum toxin injected into the IAS and topical nitroglycerin in 25 patients in each group. Healing of the fissure at one month was shown in 24 (96%) and 15 (60%) patients with a reduction in resting pressure of 26% compared with 17% respectively.

Jost and Schrank (1999a) treated 50 patients with an alternative form of botulinum toxin type A, Dysport (Ipsen Pharmaceuticals, Dublin, Ireland), in doses of 10 units or 20 units injected adjacent to the fissure margins, with a comparable healing rate of 78% by 3 months. The external sphincter tone as measured manometrically was reduced as well as the puborectalis tone, although the authors did not mention how the tone of the latter muscle was measured.

Minguez *et al.* (1999) examined 3 different doses (10 units, 15 units and 21 units) of Botox in 69 consecutive patients (groups I, II, III) with chronic anal fissures. After one month pain relief was more evident in the second and third than in the first group (74%, 100%, and 48% respectively). There was a significant reduction of MRP only in groups II and III, but the squeeze pressure decreased significantly in all three groups. The need for reinjection due to persistence of symptoms or early relapse in the individual groups was 52%, 30%, and 37% and for subsequent internal sphincterotomy which occurred from two to six months was and 17%, 19%, and 5% respectively.

Jost and Schrank (1999b) investigated the effect of repeated injections in anal fissures that relapsed or where initial treatment was ineffective. In the fissures that recurred and were treated with the same 5 unit dose, 14 (70%) of 20 healed and, of 30 that had failed initial treatment, 19 (63%) healed when treated with 10 units. This reaffirmed botulinum toxin as an effective treatment for recurring anal fissure as well as in therapeutic failure of prior toxin injections.

Maria *et al.* (2000) treated 50 patients with chronic posterior anal fissures by injection of 20 units of botulinum toxin either into each side of the posterior (group 1) or the anterior (group 2) midline. At 2 months the fissures in 15 (60%) patients in group 1 healed compared with 22 (88%) in group 2. Injection into the anterior midline also resulted in lower of resting pressures and produced early healed scars. The authors suggested that the poorer response when the injection was directed to the posterior midline could be due to destruction of myenteric nerve fibres associated with the fibrosis or ischaemia associated with posterior chronic fissures.

Lysy *et al.* (2001) randomly assigned 30 patients with chronic anal fissures that did not respond to topical isosorbide dinitrate were to either a single injection of 20 units of botulinum toxin followed by 2.5 mg topical isosorbide dinitrate thrice daily for three months or botulinum toxin alone. At six weeks the fissures healed in 10 (66%) of 15 of the former group and 3 (20%) in the latter group. The reduction in maximum resting anal canal pressure after isosorbide dinitrate was significantly greater when applied after than

before. The authors suggested that the improved potency of isorbide dinitrate on maximum resting anal pressure after botulinum toxin injection suggested a primary cholinergic tonus dominance in some patients and not as previously claimed anal sphincter insensitivity to nitrates.

As with other forms of combination therapy the optimal dosage of botulinum toxin in combination with topical nitrates was unclear. Madalinski *et al.* (2001) administered 50 to 100 units of botulinum toxin in 13 patients with fissures previously treated with topical nitrates and 25 units of botulinum toxin; seven (54%) of 13 healed, and one patient required 100 units before the fissure healed.

Brisinda *et al.* (2002) examined the effectiveness of increasing doses of botulinum toxin injected into the IAS in promoting healing in 150 patients with chronic anal fissure. Group 1 received 20 units initially and a repeat 30 units injection if the fissure persisted; group 2 had 30 units injected initially and retreatment with 50 units if necessary. One month after injection 55 (73%) in group 1 and 65 (87%) in group 2 healed; five patients in group 2 reported incontinence of flatus that resolved spontaneously after two weeks. At two months there were 67 (89%) in group 1 and 72 (96%) in group 2 with healed fissure scars. In group 1 six (8%) fissures recurred after they had healed at one month, the fissure persisted in three patients in group 2, and never healed in two other patients.

1.7.6. OTHER POTENTIAL AGENTS

The expansion of novel therapies to modulate anal sphincter tone is based on the understanding of the innervation of the IAS. Trimebutine (3,4,5-trimethoxybenzoic acid 2-(dimethylamino)-2-phenylbutylester) causes the release and modulation of peptides and has actions mediated through opiate receptors [Delvaux and Wingate, 1997]. Trimebutine 200mg thrice daily was more effective than a daily dose of 400mg of mebeverine in reducing the frequency, duration and intensity of pain attacks in patients with irritable bowel syndrome [Schaffstein *et al.*, 1990]. Ho *et al.* (1997) investigated the role of trimebutine, reducing anal sphincter spasm and thus in relieving pain after haemorrhoidectomy. In a randomised trial 80 patients who received trimebutine in suppository form showed a 35% mean reduction in resting anal canal pressure, but there was no difference with controls in the pain score at four hours after haemorrhoidectomy. The pharmacological action of trimebutine on the IAS, and its potential role in the treatment of anal fissures has yet to be defined.

There have been now numerous agents and surgical methods developed that have a role in the treatment of chronic anal fissures. Recent evidence in the form of appropriately conducted randomized trials has helped to clarify dosages, success rates, complications and recurrences. Brown *et al.* (2002) produced a management algorithm for those with persistent and recurrent fissures. It would appear that if initial conservative treatments fail surgery with selected use of endoanal ultrasound is appropriate.

1.8. GUT MOTILITY AND THE INTERNAL ANAL SPHINCTERS

Motility of a particular gut segment depends on its extrinsic and intrinsic innervation. The extrinsic innervation is from preganglionic parasympathetic and postganglionic sympathetic nerves which constitute the autonomic nervous system. Primary afferent neurones through which visceral impulses are conducted into the central nervous system, effect reflex connections with preganglionic visceral efferent neurones. Functionally and anatomically they are related to the autonomic nervous system. Enteric nerves provide the intrinsic innervation. The anal sphincter complex, which controls defaecation, consists of an inner ring of smooth muscle (the internal anal sphincter; IAS) and an outer ring of skeletal muscle (the external anal sphincter; EAS). The IAS is an involuntary muscle that maintains anal tone. It is normally in a state of tonic contraction, due partly to the intrinsic myogenic properties of the smooth muscle and partly to extrinsic neural influences (Frenckner, 1975; Frenckner and Ihre, 1976). The EAS is a voluntary, fatigable muscle, supplied by a somatic nerve that provides short-term augmentation of anal pressure to postpone defaecation.

There has been much recent clinical interest in the pharmacological manipulation of the internal anal sphincter muscle tone, based on the understanding of the intrinsic mechanisms of smooth muscle contraction and of the complex neural control of sphincter motility.

1.8.1. AUTONOMIC TRANSMITTERS AND PHARMACOLOGY

The autonomic nervous system anatomically comprises the craniosacral parasympathetic and thoracolumbar sympathetic components, and, with the enteric nervous system, constitutes the efferent innervation of every body tissue except skeletal muscle (Kuntz, 1946; Langley, 1921). Autonomic nerves are predominately efferent, but are associated with sensory visceral afferent fibres.

The original classification of the autonomic nerves, as devised by Dale (1911), was based on the release of primary transmitters acetylcholine and noradrenaline. Those neurones that synthesise and release acetylcholine are termed "cholinergic". These include almost all preganglionic efferent fibres leaving the central nervous system, all parasympathetic postganglionic and a few sympathetic postganglionic fibres. Neurones that release noradrenaline are termed "adrenergic" or more accurately "noradrenergic", and include most postganglionic sympathetic fibres. It is now known that there are many different transmitters in autonomic neurones, these include purines (Hoyle, 1996a), peptides (Hoyle, 1996b) and nitric oxide.

Cholinergic receptor subtypes, known as 'muscarinic' and 'nicotinic' were named after the alkaloids originally used in their identification. Dale noted that the cholinergic action of muscarine, when directly applied to autonomic effector tissues, was mediated by receptors at the effector cells and not by those in the ganglia. Nicotine, by contrast, was

found to stimulate autonomic ganglia. Noradrenergic receptors are simpler to define in terms of their response to certain catecholamines and are, therefore, known as adrenoceptors. Ahlquist (1948) hypothesised that catecholamines act via two distinct receptors, α or β, and this was confirmed with the use of specific antagonists. In most smooth muscles α effects are excitatory and β effects are inhibitory, phenoxybenzamine and phentolamine block α-receptors and β-receptors are blocked by propanolol.

The adrenoceptors are further subclassified according to their preferential selective response to agonists and antagonists. The subtypes of α-receptors have been identified with radiolabelled antagonists that distinguish between α_1 and α_2 receptors; for example prazocin antagonises α_1-receptors, yohimbine antagonises α_2-receptors, and dihydroergocryptine antagonises both α_1 and α_2 receptors. The intracellular effect of α_1-receptor stimulation is mediated via an increase in inositol-1,4,5- trisphosphate (IP_3), but α_2-receptor effects are mediated via a decrease in cyclic adenine-3'5'-monophosphate (cAMP). β_1 and β_2 receptors are defined by their affinities for adrenaline and noradrenaline, β_1 receptors having equal affinity for both agents, but β_2 receptors have a higher affinity for adrenaline and they respond to isoprenaline. The intracellular effect of β-receptor stimulation is mediated via an increase in cAMP. There are more divisions of adrenoceptor subtypes, and in the gut they have roles in secretion and motility. α_1-adrenoceptors are located postjunctionally on smooth muscle cells and intrinsic neurones, while α_2 adrenoceptors may be present both pre- and postsynaptically. β_1 and β_2-

adrenoreceptors are found mainly on smooth muscle cells, but the former may be present on enteric neurons (De Ponti *et al.*, 1996).

1.9. NERVOUS CONTROL OF THE INTERNAL ANAL SPHINCTER

The IAS receives its sympathetic innervation from the hypogastric pelvic plexuses. The parasympathetic innervation is from the first, second and third sacral segments via the pelvic plexus (Schuster, 1968). There seems to be some continuous excitatory sympathetic activity contributing to IAS tone but the precise interactions of the parasympathetic and enteric neurones are still poorly defined. Furthermore there is a myogenic component which contributes to normal IAS basal tone (Frenckner, 1975; Frenckner and Euler, 1975; Frenckner and Ihre, 1976; Guiterrez and Shah, 1975).

1.9.1. *IN VIVO* STUDIES

Most of the work investigating the adrenergic contribution to internal anal sphincter resting tone has been based on ideas introduced by Gaskell (1920). He suggested that the sympathetic nerves were excitatory and the parasympathetic inhibitory to the sphincter. Learmonth's (1929) investigations on anaesthetised dogs, a decade later, confirmed Gaskell's initial ideas. In these experiments, electrical stimulation of the lumbar sympathetic trunks and hypogastric nerves resulted in a contraction of the IAS. It was concluded that the IAS received some motor innervation through the lumbar sympathetic outflow. Clinical significance of these observations was given by Rankin and Learmonth (1930) who performed a limited sympathectomy in order to treat Hirschsprung's disease

and certain types of constipation. In a young anaesthetised woman, stimulation of the peripheral end of the cut presacral nerves (pre-ganglionic sympathetic nerves which divide into the hypogastric nerves) resulted in "strong clonic contractions of the sphincter followed by several weaker clonic contractions". These findings were the subjective comments of a surgical assistant with his gloved finger in the anal canal.

Shepherd and Wright (1968) investigated three normal subjects and eleven patients with Hirschsprung's disease or acquired sigmoid megacolon. They were unable to obtain simple or clonic contractions of the IAS with presacral nerve stimulation, as reported by Rankin and Learmonth (1930). Instead they demonstrated a clear inhibition of contraction of the sphincter with presacral nerve stimulation in all cases, but were unable to explain either the mechanism or the neurotransmitter involved.

Electrical stimulation of the hypogastric nerves produced consistent contractile responses in animal studies. Carlstedt *et al.* (1988) observed IAS contraction in cats, and similar contractile responses were reported in dogs (Mizutani and Nakayama, 1986) and in opossums (Shibamoto *et al.*, 1994). However, in a recent study on human subjects, Lubowski *et al.* (1987) consistently obtained decreases in IAS tone in anaesthetised patients which were reproducible using a range of stimulation frequencies and intensities. They suggested that this effect was mediated through inhibitory β-adrenergic fibres.

Frenckner and Euler (1975) examined the influence of the autonomic nerves on the IAS in man by comparing the anal canal pressure in patients after a high or low spinal

anaesthetic, or bilateral pudendal nerve blocks. These inhibit respectively autonomic and somatic supply to the sphincter complex, the parasympathetic and somatic supply, and the somatic motor nerve supply to the external sphincter muscle. They demonstrated a decrease in the anal canal pressure of 32mmHg with high spinal anaesthesia but only about 10mmHg with low spinal or pudendal nerve block. These findings reaffirm the theory that, at rest, there is a tonic excitatory sympathetic discharge to the IAS. The contribution of the parasympathetic supply is not as significant.

Penninckx' group studied the physiological responses of the IAS in cats to various pharmacological agents. Adrenergic stimulation was performed by means of successive intravenous administration of adrenaline, noradrenaline and isoprenaline, while phenoxybenzamine and propanolol were used as α- and β-adrenoceptor antagonists respectively (Kerremans and Penninckx, 1970; Penninckx *et al.*, 1973). Pressure recordings were made by means of micro-balloons placed in the rectum and anal canal; simultaneous measurements of electrical activity in the internal sphincter and rectum were also obtained. They demonstrated two types of pressure waves in the normal anal canal; 'anal pressure waves' and 'intestinal-like pressure waves'. The anal pressure waves are phasic contractions occurring at a frequency of between 8-30/min. The intestinal-like pressure waves are intermittent changes in the baseline pressure occurring at a rate of 2-5/min and usually lasting more than 10 seconds. They also showed that the anal pressure wave frequency and the level of the baseline pressure were increased by the administration of adrenaline and noradrenaline through activity of excitatory α-adrenoceptors. Attempts at defining the precise regulatory role of β-receptors were

variable; an inhibitory effect was observed in 7/16 stimulations with isoprenaline after α-receptor blockade, implying that β-receptor stimulation has an inhibitory action on basal IAS tone.

Yamato and Rattan (1990) published their findings on the role of α-receptor subtypes, based on clinical anorectal physiological measurements in anaesthetised opossums. Pancuronium bromide was used to inhibit the external sphincter, whilst the resting pressure in the anal canal, representing the IAS tone, was measured by a 7-channel catheter assembly. They demonstrated that the α_1-agonist phenylephrine caused a dose-dependent rise in the resting IAS pressure, with the more-proximal part of the anal canal producing a significantly higher response than the distal part. This response was antagonised by prazocin, an α_1- antagonist. It was not modified by the neurotoxin tetrodotoxin (TTX), supporting the concept that α_1-adrenoceptors reside on smooth muscle cells. Clonidine, an α_2-agonist, caused almost complete inhibition of IAS relaxation in response to rectal balloon distension; this response was antagonised by yohimbine. The site of action of clonidine was suggested to be proximal to the neuromuscular junction. These findings imply that activation of α_1-adrenoceptors exerts primarily excitatory effects on the IAS smooth muscle, and activation of α_2-adrenoceptors exerts an inhibitory modulatory action on the recto-anal inhibitory reflex causing suppression of reflex-mediated IAS relaxation.

1.9.2. *IN VITRO* STUDIES

Parks *et al.* (1969) described the effects of noradrenaline, adrenaline, isoprenaline, acetylcholine and nicotine on muscle strips obtained from the upper and lower parts of the human IAS. Noradrenaline caused contraction of both parts, but caused relaxation when given after exposure of the muscle strip to phenoxybenzamine, an α-receptor antagonist. This relaxation was attributed to stimulation of residual β-receptors, since this effect could be counteracted by pronethalol (a β-receptor antagonist). Isoprenaline caused relaxation of both parts of the sphincter, which was inhibited by pronethalol. The action of adrenaline on the lower IAS muscle strips was complementary to these observations as low concentrations produced relaxation by preferential stimulation of β-receptors and higher concentrations produced contractions by successive recruitment of α-receptors. Pronethalol antagonised relaxation and allowed a contraction to occur with a lower concentration, while phenoxybenzamine prevented the contraction and allowed relaxation. Fewer conclusions could be drawn about the action of nicotine and acetylcholine. Although nicotine produced relaxation of strips from the upper part of the IAS, it had no effect on the lower part and the mechanism of action could not be clarified from the data available. Acetylcholine induced contractions in most muscle strips from the upper part of the sphincter, whereas the lower part of the IAS was relatively insensitive.

Friedmann (1968) made similar observations when investigating the actions of catecholamines and nicotine on human isolated IAS muscle strips. Contractions occurred in both the proximal and distal parts of the IAS with noradrenaline, whereas there was either contraction or relaxation with adrenaline, and only relaxation with isoprenaline.

Nicotine usually caused muscle relaxation, whereas dimethylphenylpiperazinium (DMPP), a nicotinic stimulant, caused contraction. This discrepancy was thought to be due to the differing molecular sizes of the drugs; nicotine released noradrenaline close to β-adrenoceptors, and DMPP released noradrenaline near to α-adrenoceptors. Both proximal and distal segments of the anal sphincter were relatively unresponsive to acetylcholine; only minor contractions or relaxations occurred, despite the addition of the anticholinesterase physostigmine.

There had been no uniform consensus regarding the action of acetylcholine on IAS tone (Bass *et al.*, 1970; Friedmann, 1968; Parks *et al.*, 1969). Burleigh *et al.* (1979) sought to clarify the responses of isolated human IAS to cholinergic and to electrical field stimulation. The experiments reported represented data from 408 strips from 55 operative specimens; they noted that acetylcholine and bethanechol usually relaxed muscle strips; this effect was abolished by hyoscine and antagonized to a varying degree by tetrodotoxin. Sphincter muscle was also relaxed by electrical field stimulation of intrinsic nerves; this response was blocked by tetrodotoxin but unaffected by hexamethonium, hyoscine, or propranolol.

The effects of acetylcholine were mediated via by muscarinic receptors on non-adrenergic non-cholinergic (NANC) inhibitory enteric neurones [Buckley and Caulfield, 1992]. Since the predominant inhibitory transmitter of NANC transmission in the IAS has now been identified as nitric oxide, it seems that acetylcholine relaxes the IAS by stimulating nitric oxide synthesis. They also demonstrated that bethanecol, a cholinergic agonist that

selectively stimulates muscarinic receptors, relaxed IAS strips. This is consistent with *in vivo* observations by Gutierrez and Shah (1975) where an infusion of bethanecol lowered basal anal pressure by stimulation of inhibitory muscarinic receptors. Electrical field stimulation always caused relaxations, by stimulation of these same NANC enteric inhibitory nerves. These relaxations were unaffected by hyoscine, propanolol, or hexamethonium which prevented relaxations to acetylcholine, isoprenaline, and DMPP respectively. However the response was prevented by tetrodotoxin, which blocks nerve action potential propagation, demonstrating the neural action of electrical field stimulation.

1.10. THE ENTERIC NERVOUS SYSTEM

The enteric nervous system was defined by Langley (1921) as a third division of the autonomic nervous system, which receives inputs from the sympathetic and parasympathetic divisions. It exists as a separate entity in the gastrointestinal tract and comprises the internal and external submucosal plexuses (Meissner's and Schbadach's plexus respectively), the myenteric plexus (Auerbach's plexus) and the subserosal plexus - all of which are ganglionated (Furness and Costa, 1987). There are also non-ganglionated plexuses such as the deep muscular plexus and networks in the submucosa and lamina propria of the mucosa. Langley demonstrated histological differences between enteric neurones and neurones of other autonomic ganglia. He also showed that the connections between fibres from the central nervous system and the enteric neurones

differ from those between the central nervous system and other peripheral ganglia. Specifically he noted the great discrepancy in number between the few fibres in nerves running to the intestine and the large populations of enteric nerve cells. In addition he pointed out the existence of complex local reflex pathways within the enteric nervous system, which function as afferent, efferent or interneuronal pathways. These constitute all the nervous components necessary for co-ordinated activities within the intramural plexuses, as exemplified by the recto-anal inhibitory reflex.

Gowers (1877) demonstrated that air injected into the rectum caused a rapid fall in anal canal pressure, due to relaxation of the IAS, followed by a steady return to the initial resting pressure. This response is now known as the "rectoanal inhibitory reflex" (RAIR). A normal reflex is elicited in patients with cauda equina lesions (Denny-Brown and Robertson, 1935), and in individuals with spinal cord transection (Burleigh and D'Mello, 1983), which clearly indicates that it is entirely intrinsic and independent of higher neural centres. The reflex is absent in Hirschsprung's disease, which is characterised by an absence of ganglia in a variable length of the rectum and colon. It may be abolished temporarily after restorative rectal resection (Schuster, 1963), but in the long term is restored by regeneration of intramural neurones across the anastomosis (Lane and Parks, 1977). Sphincter function is often dependent on adjacent gastrointestinal movement, and its tone altered through the release of neurotransmitters. These are specific for each different gut sphincter (Bennett and Whitney, 1966).

Numerous enteric neurotransmitters have now been identified, several of which may be released from the same neurone. The release of these transmitters may be affected by pre- and post-synaptic neural modulation.

1.10.1. ENTERIC INHIBITORY NEUROTRANSMITTERS

The existence of enteric inhibitory neurones with unknown transmitters which do not rely on adrenergic or cholinergic transmitters, labelled non-adrenergic nor non-cholinergic (NANC), was first suggested by the work of Langley and Anderson (1896). The characterisation of the neurotransmitters involved came nearly seventy years later. Burnstock *et al.* (1963) recorded hyperpolarisations in intestinal muscle during stimulation of the intrinsic nerves in the presence of atropine and the adrenergic neurone blocker bretylium. The block of these hyperpolarisations by tetrodotoxin suggested they were inhibitory junction potentials in response to stimulation of NANC nerves (Burnstock *et al.* 1964; Bennett and Whitney, 1966). Inhibitory junction potentials are electrical events that underlie the relaxation of muscle; they are the hyperpolarising response of smooth muscle in response to nerve stimulation and, as such, they cause an inhibition of neurotransmission by inhibiting action potential propagation.

Circular muscle strips from the IAS relax via NANC inhibitory nerves when exposed to electrical field stimulation. Speakman *et al.* (1990) found that 4/5 control preparations

showed a small contraction followed by relaxation. The contraction was abolished by the α-adrenoceptor blocker phentolamine.

There has been an extensive search for the intramural NANC transmitter responsible for inducing the IAS relaxation seen with rectal distension. The transmitters noradrenaline, acetylcholine, prostaglandins E_2 and $F_{2\alpha}$, 5-hydoxytryptamine (5-HT) and dopamine have already been discounted as mediators for the RAIR (Burleigh *et al.* 1979). Over the last two decades work has concentrated on the function of adenosine triphosphate (ATP), vasoactive intestinal peptide (VIP), and nitric oxide (NO) and their role in mediating the RAIR, as they are known to act together in mediating enteric inhibitory co-transmission in other areas of the gut.

Adenosine Triphosphate (ATP)

There is evidence to suggest that ATP is a neurotransmitter in the enteric nervous system, and the release of ATP through electrical stimulation of enteric nerves has been measured. The pharmacological responses to ATP can be blocked by purinoceptor antagonists such as suramin or reactive blue 2 (Hoyle, 1996a). Although reliable histochemical stains for ATP are not known, a fluorescence technique involving quinacrine, which forms complexes with ATP, has been developed. Quinacrine is known to bind to compounds with transmitter-like properties, though its efficacy for neurones that have a high ATP content is uncertain. Despite its inhibitory action in other parts of the gut, there is no evidence of such a role for ATP in the IAS (Burleigh *et al.* 1979).

Vasoactive Intestinal Peptide (VIP)

VIP has been identified as an inhibitory transmitter mediating lower oesophagealrelaxation (Biancani *et al.* 1984). VIP neurones have been localised in the IAS (Alumets *et al.* 1978), and VIP is released in the venous effluent from the rectum following neural stimulation (Andersson *et al.* 1983). Biancani *et al.* (1985) demonstrated that preincubation with VIP-antisera inhibited relaxation of IAS strips in response to both exogenously applied VIP and electrical field stimulation. Nurko *et al.* (1989) used specific VIP antagonists to demonstrate VIP as a NANC transmitter. The role of other co-transmitters cannot be discounted since VIP antagonists fail to inhibit completely IAS relaxation in response to lower levels (0.5Hz) of neural field stimulation.

Nitric Oxide (NO)

From their work on isolated strips of opossum IAS, Rattan and Chakder (1992) suggested the possibility of NO as an inhibitory neurotransmitter of NANC nerves in the human IAS. First, they showed that NO caused a tetrodotoxin-resistant relaxation in these strips. Second, they showed that L-N^G-nitro-arginine, a NO synthesis inhibitor, caused a stereoselective suppression of the fall in IAS tension due to electrical field stimulation; this effect was counteracted by the application of the endogenous precursor of NO, L-arginine. This latter finding is consistent with Burleigh's observations that L-N^G-nitro-arginine reduced NANC relaxations of the human gut (Burleigh, 1992). Rattan suggested that NO worked in conjunction with VIP, since L-N^G-nitro-arginine also suppressed the fall in resting tension of IAS in response to VIP (Rattan and Chakder, 1992).

In further work on opossums, Chakder *et al.* (1993) demonstrated, by chemiluminescence, that NO was released in response to NANC stimulation. O'Kelly *et al.* (1993) in similar experiments on isolated human IAS strips, demonstrated a concentration dependent inhibition of IAS relaxation to electrical field stimulation by application of L-N^G-nitro-arginine, or the weaker competitive analogue of L-arginine, N-monomethyl-L-arginine. However the inhibitory effect of L-NNA was complete, disputing the involvement of other transmitters.

Using nitric oxide synthase immunoreactivity, and nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase histochemistry, O'Kelly *et al.* (1994) presented morphological data suggesting that, in the human anorectum, NO synthase-containing neurones and their processes have the appropriate characteristics to serve as inhibitory motor nerves between the rectum and anal canal. Bult *et al.* (1990) demonstrated release of NO from NANC nerves. In a guinea-pig model, Stebbing (1998) using retrograde neuronal tracing and enzyme histochemistry, described direct anatomical evidence of an efferent descending nitrergic rectoanal pathway appropriate to mediating the motor response of the RAIR. NO activates soluble guanylate cyclase, thereby increasing production of cyclic guanosine-3'5'-monophosphate (cGMP) and relaxation of smooth muscle. NO is important in the RAIR, but other transmitters may have a role.

1.11. SMOOTH MUSCLE CONTRACTION AND MAINTENANCE OF TONE

Autonomic and enteric neural stimulation regulates smooth muscle contraction in the IAS. Contraction of the IAS translates clinically into an increase in anal tone.

Muscle contraction is regulated by changes in cytosolic calcium (Ca^{2+}) levels. Smooth muscle differs from striated muscle in the way that its contractile elements are activated. The two major contractile proteins in both muscle types are actin and myosin, which are structural components of the thin and thick filaments respectively.

In smooth muscle, Ca^{2+} regulation is affected via the various regulatory proteins including the myosin light chains, calmodulin, caldesmon, and calponin. Ca^{2+} release into the myoplasm occurs either by entry through voltage-gated or receptor-operated channels or by release from the sarcoplasmic reticulum via inositol triphosphate (*fig. 1.3.*). Ca^{2+} binds to calmodulin which then activates myosin light chain kinase, thus inducing phosphorylation of the regulatory light chains of myosin. When actin has been activated, by caldesmon, phosphorylated myosin then attaches to actin, this results in the production of tension. Dephosphorylation of myosin induces either relaxation or may allow cross-bridges to enter the "latch state", which is defined as a state of prolonged contraction in the smooth muscle with a low frequency of cross-bridge cycling. The precise mechanisms that are responsible for this maintenance of smooth muscle tone are still not entirely known.

Relaxation of smooth muscle occurs when there is a resulting decrease in cytosolic Ca^{2+}. This is effected through cellular mechanisms similar to those for contraction. *In vitro* studies of porcine IAS have shown that tone and spontaneous activity depend on the extracellular Ca^{2+} and flux across the cell membrane, whereas agonist-induced contractions were depend mainly on Ca^{2+} release from the intracellular sarcoplasmic reticulum [Cook *et al.* 1999b]. Understanding the mechanisms that maintain tone and muscle contraction in relation to cellular Ca^{2+} could aid the treatment of smooth muscle contractile abnormalities.

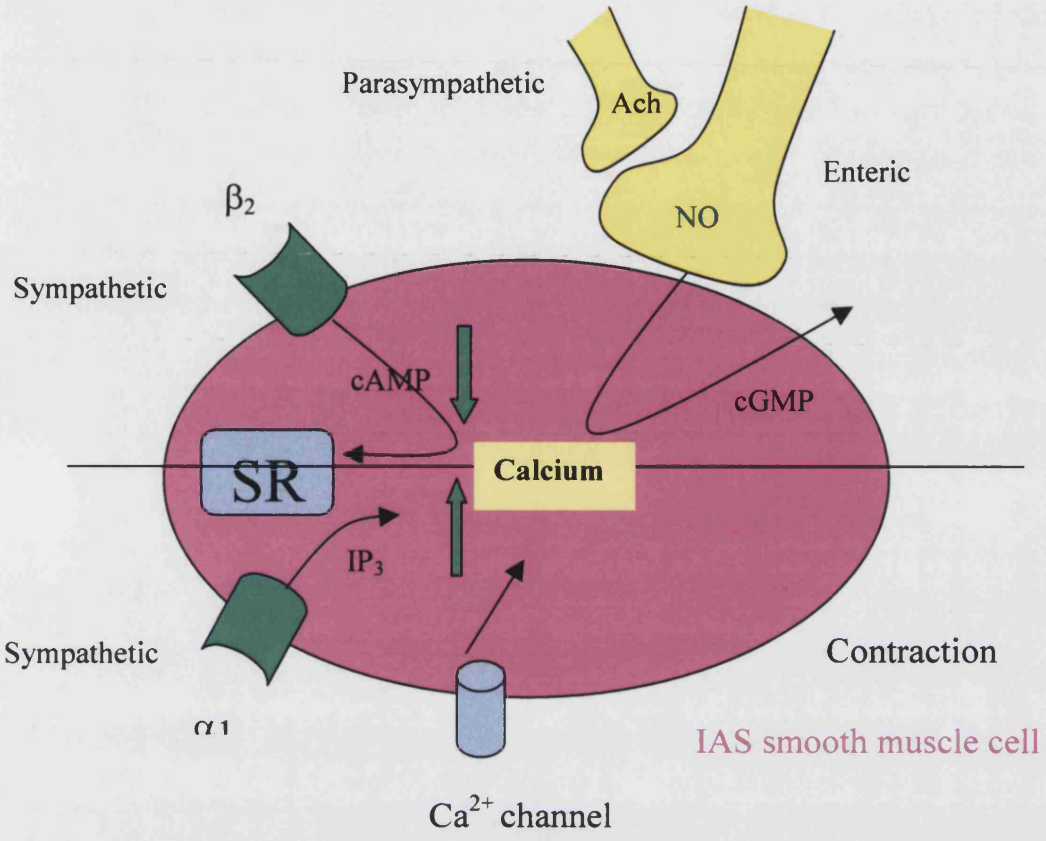


Figure 1.3. Internal anal sphincter (IAS) smooth muscle cell contraction is dependent on a rise in cellular calcium (Ca^{2+}). This can be effected by direct influx of calcium through membrane Ca^{2+} channels or by stimulation of α_1-adrenoceptors. The latter pathway results in release of Ca^{2+} from the sarcoplasmic reticulum mediated via inositol-1'4'5'-triphosphate (IP_3). Relaxation is induced by antagonising membrane Ca^{2+} channels or α_1-adrenoceptors. Stimulation of β_2-adrenoceptors results in a return of Ca^{2+} to the sarcoplasmic reticulum, mediated through cyclic adenine-3'5'-monophosphate (cAMP). Stimulation of nitric oxide (NO) containing non-adrenergic non-cholinergic (NANC) enteric neurones induces a cyclic guanosine-3'5'-monophosphate (cGMP) mediated decrease in cellular Ca^{2+}. Muscarinic nerve stimulation, through the release of acetylcholine (Ach) promotes the synthesis of NO.

CHAPTER TWO

AIMS OF THESIS

2.1. INTRODUCTION

Idiopathic chronic anal fissures continue to cause significant morbidity in a relatively young population. It is generally thought that fissures develop from a traumatic insult to the anoderm, commonly in the posterior midline, and the subsequent anal spasm that develops compromises the vascular perfusion to this segment. This continued vascular ischaemia perpetuates the chronicity of a fissure. It is anal spasm that induces the pain patients present with. Whilst traditional therapeutic modalities for treatment of chronic anal fissures have been constantly re-evaluated newer non-surgical options have developed, with a common underlying objective. This is to effectively reduce maximum resting anal canal pressure sufficiently for healing to occur *without* a permanent detrimental effect on sphincter function. Sustained reduction in anal canal pressure after therapeutic benefit that is achieved may induce incontinence in the long term.

Accounts of surgical procedures extend from the early nineteenth century; these include anal dilatation, lateral sphincterotomy, midline posterior sphincterotomy, fissurectomy, and anal advancement flaps. Manual anal dilatation causes uncontrolled tearing of the anal sphincter complex and the shearing forces result in fragmentation of the sphincters that lead to incontinence in 12.5% of subjects, although not all patients with sphincteric defects experience incontinence. Controlled digital dilatation of the anus under neuromuscular blockade has been shown to reduce the incontinence rate to 3.8%. The midline posterior sphincterotomy with or without fissurectomy has been largely abandoned because of the keyhole deformity it creates that is responsible for faecal

soilage. An advancement flap is reserved for patients with chronic anal fissures associated with normal or low resting pressure without anal spasm and after failed lateral sphincterotomy. Manometry and endoanal ultrasound are being used more often in patient selection.

Farouk *et al.* (1998) in a retrospective analysis defined the treatment strategy for chronic anal fissure between January 1990 and December 1996. Of 221 treated 209 underwent surgery. Manual dilatation of the anus was performed in 21 (10%) patients and was abandoned in 1995. Lateral internal sphincterotomy, the principal treatment, was performed in 183 (88%) patients; five female patients (2%) with a sphincter defect identified by anal manometry and endoanal ultrasound examination underwent anal advancement flap. Since 1996 15 (7%) were treated by topical GTN as the first line of treatment, in nine the fissure healed, three were relieved of pain without healing of the fissure, and three proceeded to lateral sphincterotomy.

Nelson *et al.* (1999) undertook a meta-analysis of operative methods for fissure-in-ano that looked at two end points, namely non-healing and flatus incontinence. These included anal stretch, open or closed lateral sphincterotomy, posterior midline sphincterotomy, and dermal flap coverage of the fissure. There were 17 reports encompassing 2727 patients. The authors concluded anal stretch and posterior midline internal sphincterotomy should be abandoned and either the open or closed technique of lateral anal sphincterotomy was equally efficacious.

The role of open lateral sphincterotomy under sedation and local anaesthesia after failed conservative therapy was investigated in 2108 patients by Argov *et al.* (2000) with a follow up period ranging from 4 to 20 years. The short-term complication rate was 3%; this included temporary incontinence to flatus and liquid stool, usually subsiding within 2 to 3 months; haematoma and wound infection; and urinary retention. There was 1% recurrence rate, occurring usually within the first year that necessitated re-sphincterotomy on the opposite side. There was not a single case of permanent incontinence.

Since Langley's (1896) description of the autonomic nervous system much progress has been made in defining the neural pathways and their neurotransmitters that has led to better understanding of the neuromyogenic properties of the human internal anal sphincter and the role of pharmacological agents in the treatment of anal fissures. Nitric oxide is the predominant transmitter of the non adrenergic non cholinergic enteric neurones. When stimulated this pathway causes marked relaxation of the internal anal sphincter. GTN as a nitric oxide donor when applied topically (in a paraffin base at a concentration of 0.2%) reduces anal sphincter tone and has been used as a first line agent and induces healing of chronic anal fissures in two-thirds of patients. However headache has been its main side effect that has influenced patients' compliance and hence its therapeutic use. Isosorbide dinitrate, which acts in a similar manner, has been used with equal efficacy although headaches are still common with its use. The contractility and maintenance of IAS smooth muscle tone is dependent on calcium. Diltiazem ointment (2%) prevents flux of calcium through L-type membrane channels and induces a reduction in maximum resting anal canal pressure. This agent is being used in preference

to topical GTN with comparable results without causing headache or other side effects. Botulinum toxin is effective in the treatment of chronic anal fissures. It is believed it acts on the IAS by modulating the sympathetic neurones. Its use has been widely evaluated but its main drawback is its mode of delivery into the sphincters by injection, which besides being unpleasant for patients is a cause of sepsis and bleeding that complicate the procedure. The effect of oral indoramin, an α-adrenergic antagonist, and oral salbutamol, a β-adrenergic agonist on maximum resting anal canal pressure has been examined. Both agents cause a reduction in both patients with chronic anal fissures and healthy volunteers. However these observations have not been validated. The relaxant responses of these two drugs suggest up-regulation of β-adrenoceptors in patients with chronic anal fissures.

The work in this thesis takes into account several unresolved issues with respect to the pathophysiology of chronic anal fissures and the therapeutic role of pharmacological agents. GTN has been well evaluated in the treatment of chronic anal fissures. Diltiazem is emerging as an alternative and safer option and deserves similar scrutiny. While botulinum toxin is probably the most effective non-surgical modality its unacceptability seems to be related to its mode of delivery that if improved may promote its application. The search for the optimal pharmacological agent will continue and the potential effect of drugs on the sympathetic adrenergic receptors has not been adequately explored. A suitable animal model for human IAS would provide means of exploring the effect of pharmacological agents on the IAS.

2.2. AIMS

Therefore the work presented in this thesis is in two sections: clinical applications and experimental work.

2.2.1. CLINICAL APPLICATIONS

1. Topical diltiazem in treatment of anal fissures

Diltiazem is showing promise as an effective agent in the treatment of anal fissures. The manometric response to this drug in relation to healing during and after cessation of treatment has not been previously investigated. Although some studies have attempted to define clinical prognostic indicators there is not sufficient evidence to draw out firm conclusions. An open trial with 2% diltiazem gel in 33 patients was conducted, and the healing and manometric responses measured during a six month period. The relationship of the clinical parameters to healing was examined.

2. Novel delivery of botulinum toxin for treatment of anal fissure

There is evidence that Botox (botulinum toxin, Allergan, UK) is effective treatment of chronic anal fissures. It is likely that direct injection into the sphincter complex may influence its acceptability by both clinicians and patients. The needle-less J-tip© syringe uses a prefilled carbon dioxide canister to eject its contents through a narrow port at its tip. It was essential to find out whether the therapeutic agent reached the target organ. This was indirectly determined by

estimating the manometric and healing response in 10 patients with chronic anal fissures. It was appropriate to conduct studies in porcine anal sphincter to establish the optimal angle of injection for the Botox to infiltrate the IAS adequately.

3. Sympathetic modulation of the IAS

The effect of oral indoramin and salbutamol on IAS tone was re-examined with particular reference to side effects. A topical preparation of whichever drug that reduced maximum resting anal canal pressure with least side effects was formulated and tested in different doses in healthy volunteers.

2.2.2. *IN-VITRO* EXPERIMENTAL WORK

1. Validation of an animal model of human IAS tissue

Human IAS is not easily available for investigation of its neuromyogenic features. An animal model with physiological similarities was necessary. The physical characteristics of guinea pig tissue was initially examined and its responses to histamine, atropine, methoxamine, acetylcholine and electrical field stimulation (EFS) were quantified. A porcine model was then studied in a similar manner; phenylephrine was used to modulate the tone of the muscle. The effects of nitric oxide donors and antagonists, purinergic antagonists, α-adrenoceptor antagonists and botulinum toxin were also investigated.

2. Investigation into the properties of porcine IAS muscle

Most chronic anal fissures are associated with hypertonia, yet a reduction in maximum resting anal canal pressure with topical agents in these patients may not heal the fissure. The changes in the myogenic behaviour of IAS tissue as its tone was increased with phenylephrine was investigated. It was an attempt to find an explanation for the ineffectiveness of reduction in tone in the healing of some chronic fissures associated with hypertonia.

CHAPTER THREE

TOPICAL DILTIAZEM IN TREATMENT OF ANAL FISSURES

3.1. INTRODUCTION

Lateral sphincterotomy allows healing of chronic anal fissures by reducing the MRP. Boulos and Araujo (1984) demonstrated a reduction in MRP after subcutaneous or open sphincterotomy. This magnitude of reduction is sustained, as Chowcat *et al.* (1986) has demonstrated in 28 patients one year after surgery. Whilst effective in healing fissures Khubchandani *et al.* (1989) report on long-term clinical sequelae of internal sphincterotomy was alarming as 35.1% of 829 patients complained of lack of control of gas ranging from "sometimes" to "frequently", furthermore 56 (6.7%) expressed dissatisfaction with the result. Nyam and Pemberton (1999) found a 3% incontinence rate among 585 patients after sphincterotomy at a mean follow up of 72 months. While these reports and the endosonographic studies that emerged from Sultan *et al.* (1994) and Farouk *et al.* (1998) called for careful patient selection and precision in technique, investigators looking for a safer option explored the feasibility of chemical agents to treat chronic anal fissures that would not jeopardise sphincter function.

O'Kelly *et al.* (1993) discovery that nitric oxide was a significant neurotransmitter that induced relaxation in the IAS prompted clinical interest. Initial trials by Loder *et al.* (1994) using 0.5% and 1% GTN and subsequent work by Lund *et al.* (1996) using 0.2% GTN demonstrated the efficacy of GTN in healing fissures. The success with this agent was offset by headaches, which is its main side effect reported in at least 20% of patients [Gorfine (1995a), Lund and Scholefield (1996)] and alarmingly in 72% of patients investigated by Carapeti *et al.* (1999a). Dorfmann *et al.* (1999) also reported headaches

in 21 (78%) of 27 patients on GTN that were severe in 2 (10%) patients who as a result discontinued therapy. Palazzo *et al.* (2000) treated 45 patients treated with GTN, 84% had headaches and 11% discontinued treatment. Richard *et al.* (2000) used 0.5% GTN in 11 and 0.2% GTN in 33 patients. In total, of the 44 patients assigned to GTN, 9 patients discontinued GTN because of headaches (8) or significant postural hypotension (1); 5 (55%) were on 0.5% GTN, and 4 (45%) were on 0.25% GTN.

The healing rates reported in most recent trials ranged from 50 to 75%; in 29 (49.2%) of 59 patients after a 4 week course of 0.2% GTN [Altomare *et al.* (2000)], in 20 (50%) of 40 patients by 12 weeks [Hasegawa *et al.* (2000)] and in 33 (73%) of 45 patients at 6 weeks, although in this series a higher dose (0.5%) was prescribed [Palazzo *et al.* (2000)].

Pitt *et al.* (2001a) showed that whilst the recurrence rate of anal fissures after successful treatment with GTN varies from 40% to 70%, of 26 (40.6%) of 64 patients with fissures healed initially with GTN, 12 (46.2%) recurred within 32 weeks. Graziano *et al.* (2001) in a long-term series, of a median follow up of 39 weeks, showed that whilst 0.25% GTN healed 75% of chronic fissures and 83% of acute fissures, 67% recurred by 9 months. Richard *et al.* (2000) identified among 13 (29.5%) of 44 patients with healed fissures with GTN 5 (38%) recurrences by 6 months.

Nifedipine and diltiazem are calcium channel antagonists that prevent calcium influx through membrane channels. This diminishes the tone of the IAS, and facilitates the

healing of anal fissures by reducing the MRP and improves vascular perfusion of the anoderm.

Chrysos *et al.* (1996) demonstrated that 20mg oral nifedipine reduced the MRP significantly in 10 patients with non prolapsing haemorrhoids and/or anal fissures and 10 volunteers by 32% and 24% respectively. Antropoli *et al.* (1999) showed that 0.2% topical nifedipine gel healed acute anal fissures in 141 patients after 3 weeks with a reduction in MRP of 30% from 72.5 mmHg to 50.5 mmHg. Cook *et al.* (1999a) found an oral dose of 20mg nifedipine reduced the MRP significantly by 36% from 102 to 65 cmH_2O in 15 patients with chronic anal fissures, 3 (20%) healed by 6 weeks and 9 (60%) after 8 weeks.

Carapeti *et al.* (1998a, 1998b, 1999b) demonstrated that a single dose of oral diltiazem significantly lowered the mean MRP by 21%, from 97 to 77 cmH_2O, and a topical application of 2% diltiazem significantly lowered the mean MRP by 28% from 105 to 76 cmH_2O, an effect that lasted 3 to 5 hours after application, without side effects. Higher concentrations produced no additional effect. Carapeti *et al.* (2000) treated 15 patients with chronic anal fissures with 2% diltiazem gel thrice daily for 8 weeks. The starting MRP was elevated in 14 patients (>120 cmH_2O) and one patient had a normal resting pressure. At 8 weeks the fissures healed in 10 (67%) patients, of the remaining 5 with unhealed fissures one was asymptomatic and had a normal MRP and 4 had internal anal sphincterotomy. There was no significant difference in the pre-treatment MRP between responders (median 120; range, 85-156 cmH_2O) and nonresponders (median 136; range,

110-140 cmH_2O) or in the post-treatment MRP between responders (median 88; range, 80-104 cmH_2O) and nonresponders (median 94; range, 82-142 cmH_2O). In the whole group there was an overall significant reduction in mean MRP but the anodermal blood flow was unaltered.

Knight *et al.* (2001) in a prospective study of 71 patients with chronic anal fissures treated with 2% topical diltiazem gel noted in 47 (73%) of 64 who attended the first review the fissures healed, of the 17 that did not heal 12 had a second course of diltiazem of whom 8 healed. At a median follow up of 32 (range 14-67) weeks 27 (66%) of 41 remained asymptomatic, 7 had mild symptoms without recurrence of the fissure, and 7 had a fissure recurrence of whom one underwent a lateral sphincterotomy, 3 healed with topical GTN, and 3 with further topical diltiazem gel.

Jonas *et al.* (2001b) investigating the role of oral diltiazem as a potential treatment for anal fissures randomised 24 patients to oral (60mg) and 26 to topical 2% diltiazem twice daily for eight weeks. The mean MRP fell significantly after the first dose in both groups; by 15% from 95 to 81 cmH_2O in the oral treatment group; and by 23% 102 to 79 cmH_2O in the topical treatment group. At 8 weeks the fissures healed in 9 (38%) patients who were treated with oral diltiazem compared with 17 (65%) who had received topical treatment.

There have been few studies on the long term results of topical diltiazem treatment. DasGupta *et al.* (2002) followed up 23 patients with fissures using a thrice-daily 2%

topical diltiazem with symptoms ranging from a median of 6 (range 2-36) months. Fissure healing was seen in 11 (48%) of the group by a median follow up of 8 weeks; the 12 remaining patients either underwent lateral sphincterotomy, used GTN or declined further intervention. Griffin *et al.* (2002) in a long-term follow up study of 50 patients initially presented by Jonas *et al.* (2001b) contacted 35 with a median follow-up period of 31 (range 27-37) months. Of 17 that responded 7 (41%) reported subsequent anal symptoms after a median of 6 (range 1 to 35) months.

Kocher *et al.* (2002) randomised 29 patients with chronic anal fissures for treatment with GTN and 31 with diltiazem. After 6-8 weeks 21 (72%) of the GTN group experienced side effects, the most common being headaches, compared with 13 (42%) of the diltiazem group. 3 patients in the diltiazem group discontinued treatment because of perianal itching or pruritis. At 8 weeks 25 (86%) of 29 in the GTN group healed or improved compared with 24 (77%) of 31 patients in the diltiazem.

A chronic anal fissure is often not clearly defined in the reported results of different treatments. The clinical signs associated with chronic anal fissures include visible internal sphincter fibres, the presence of a sentinel pile, and a fibroepithelial polyp [Keighley and Williams, 1993]. Few studies have addressed the relevance of these features to the result of treatment by sphincterotomy or topical agents. Pitt *et al.* (2001a) in a Cox model multivariate analysis in 64 patients with chronic anal fissures treated with topical GTN to determine prognostic factors for healing demonstrated that a history of a fissure exceeding 6 months and the presence of a sentinel pile were associated with

failure of the fissure to heal. The presence of a sentinel pile was also associated with a higher recurrence rate, and therefore poor long-term healing. The authors suggested that patients with sentinel piles should be informed that topical GTN therapy was more likely to fail, and earlier sphincterotomy was advised.

The aims of this study were to determine in patients with chronic anal fissures the clinical and manometric response to topical diltiazem over a six-month period, with reference to the manometric patterns in responders and non responders. There have been no clinical studies that have assessed manometric responses beyond two months of treatment. The significance of duration of symptoms and the clinical features of the fissure in relation to the response and healing is also discussed.

3.2. METHODS AND PATIENTS

Thirty three consecutive patients were enrolled for this study. At initial evaluation of the patients' symptoms and their duration, the presence of a fissure, a sentinel pile and anal canal spasm were noted. All patients and, for comparison, 10 control subjects in whom two measurements were taken at alternate days had anal canal manometry.

The patients were treated with 2% topical diltiazem ointment (SLA Pharma, Herts, UK) thrice-daily. They were instructed to apply to the anal verge about one inch expressed from the tube for 2 months and this was repeated if the fissure remained unhealed.

At 2 monthly intervals until 6 months the patients had clinical and manometric evaluation. At each visit the presence or absence of symptoms was recorded and clinical examination of the anal canal when the patient was pain free and had no anal spasm included proctoscopy to assess healing defined as re-epithelialisation of the anal mucosa at the site of the fissure. Anal manometry was performed as described in *Appendix 1*, the results expressed in cmH_20 and the combined results expressed as a median value with ranges. The data is graphically presented (Prism 3.0, Graphpad, San Diego) expressing the median values with interquartile ranges. Comparison of the MRP values was conducted using the paired or unpaired *t test* after analysing data for normality.

3.3. RESULTS

The results are shown in *Appendices 2-10.* There were 17 males and 16 females with median age of 33 (range 27-81) years with history of pain in 32 and rectal bleeding in 29 for a median duration of 5 (1-36) months. On examination in all a fissure was confirmed, 19 (57.6%) had anal spasm and 22 (66.6%) had sentinel piles. The control group included 5 males and 5 females with a median age of 28 (range 21-38) years. The sex and age distribution in patients and controls were similar (*P*=NS). At the start of the study all 33 patients attended and again at 2 months but at 4 and 6 months more patients were cured or were reluctant to undergo manometry again *(fig.3.1).*

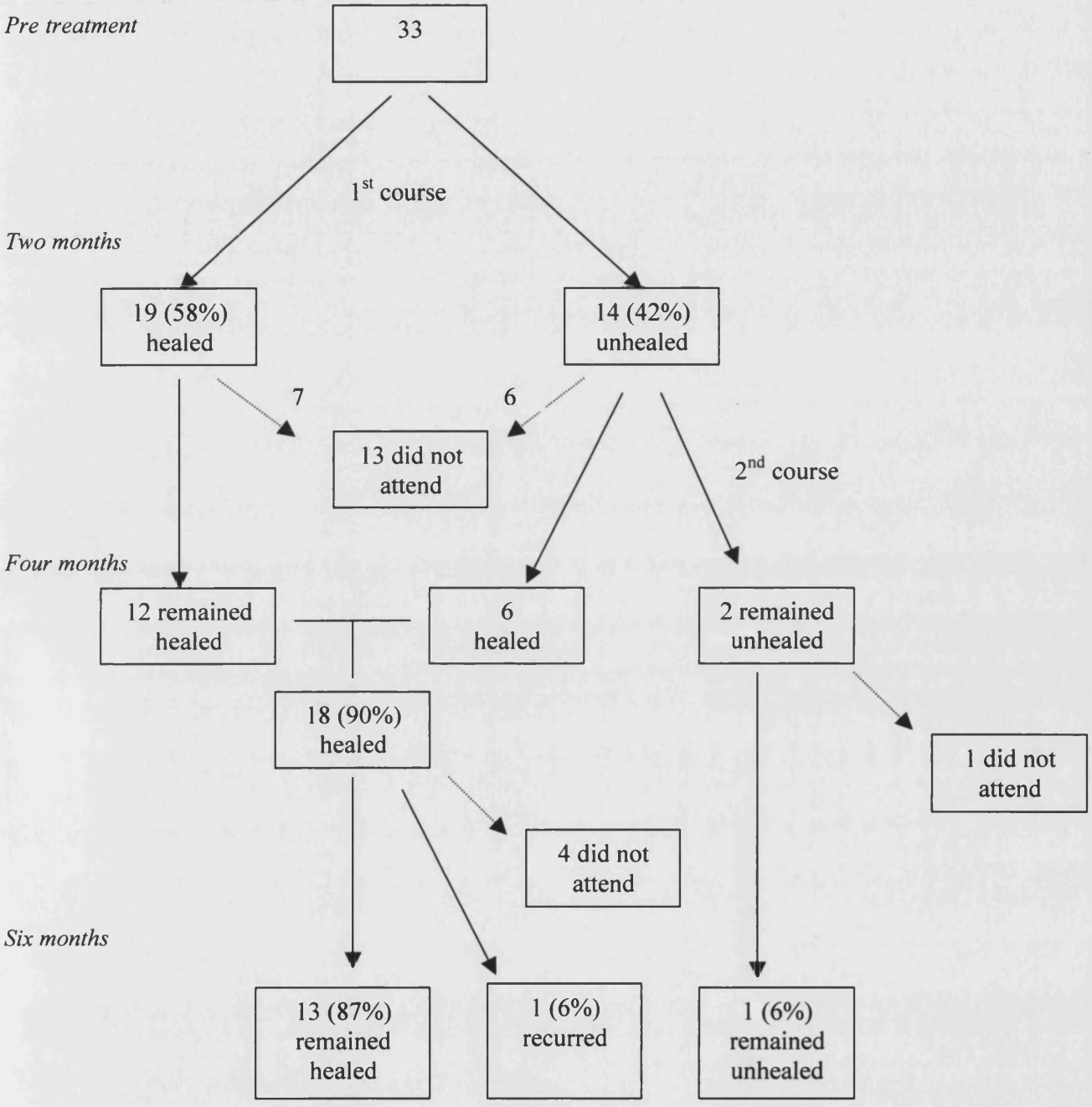


Figure 3.1. Schematic representation of the follow up of patients with chronic anal fissure

Pre treatment

The age, sex distribution and MRP are shown in *Appendices 2 and 3*. The median MRP in the 33 patients [110 (77-227)] was significantly higher than in the 20 healthy volunteer subject readings [88 (46-175) cmH_2O] ($P<0.05$). *(fig. 3.2.)*.

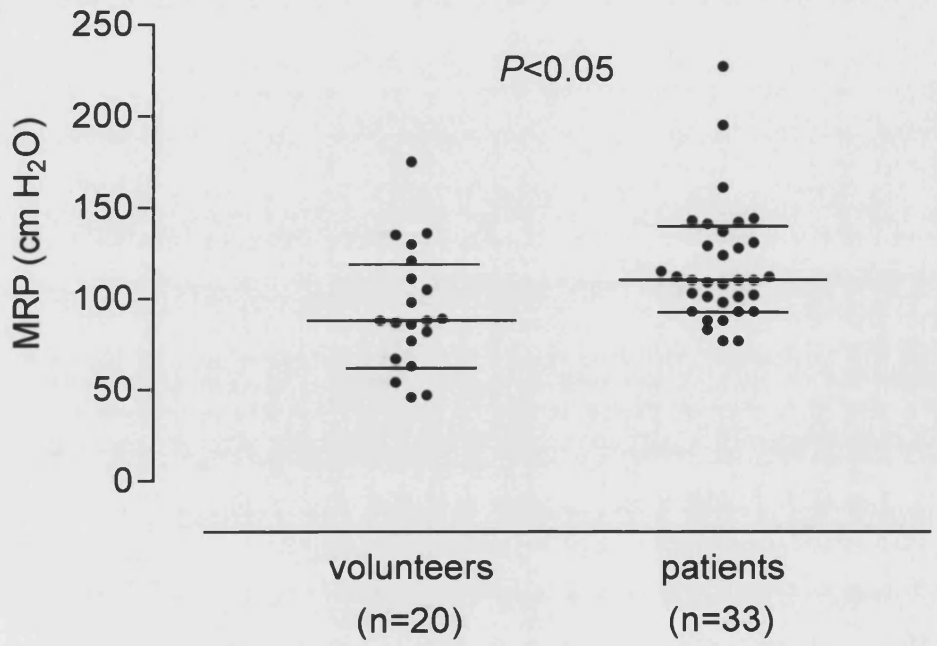


Figure 3.2. Distribution of the MRP in 20 controls and 33 patients with chronic anal fissures prior to treatment with topical 2% diltiazem. The horizontal lines represent the median values with interquartile ranges.

Assessment at 2 months

The results are shown in *Appendices 4 and 5*. The fissures healed in 19 (58%) patients and 14 remained unhealed; 18 in the healed group and all of the 14 in the unhealed group presented with pain on defaecation; and 17 in the healed and 12 in the unhealed group presented with bleeding per rectum. After 2 months all with healed fissures were asymptomatic while in the unhealed group 4 patients still had pain on defaecation, 3 had rectal bleeding and the remaining 7 were asymptomatic. There was a significant reduction of 25 % in the MRP in all 33 patients [110 (77-227) v 83 (53-147) cm H_2O, *P < 0.0001*] *(fig. 3.3.)*.

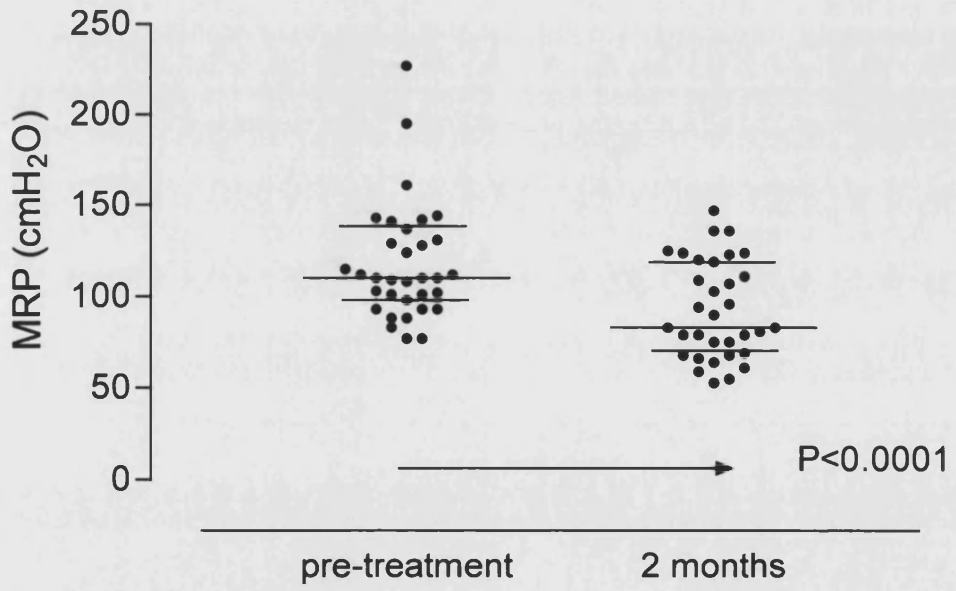


Figure 3.3. The median MRP in 33 patients with chronic anal fissures after 2 months treatment with topical 2% diltiazem. The horizontal lines represent the median values with interquartile ranges.

In the 19 patients that healed the MRP was significantly reduced by 26% [110 (77-195) v 81 (53-147) cm H_2O, *P<0.0001*] *(fig. 3.4.)*. In the 14 patients with unhealed fissures the reduction of MRP was 17%, and this was also significant [111 (77-227) v 92 (56-136) cmH_2O, *P<0.01*] *(fig. 3.4.)*. The MRPs in the groups of patients with healed and und unhealed fissures prior to treatment was not significantly different [110 (77-195) v 111 (77-227) cmH_2O, *P*=NS], in each group the MRP was significantly higher than in controls (*P*<0.05). After treatment the MRP in each group did not differ significantly [81 (53-147) v 92 (56-136) cmH_2O, *P*=NS] and was similar to the MRP in controls.

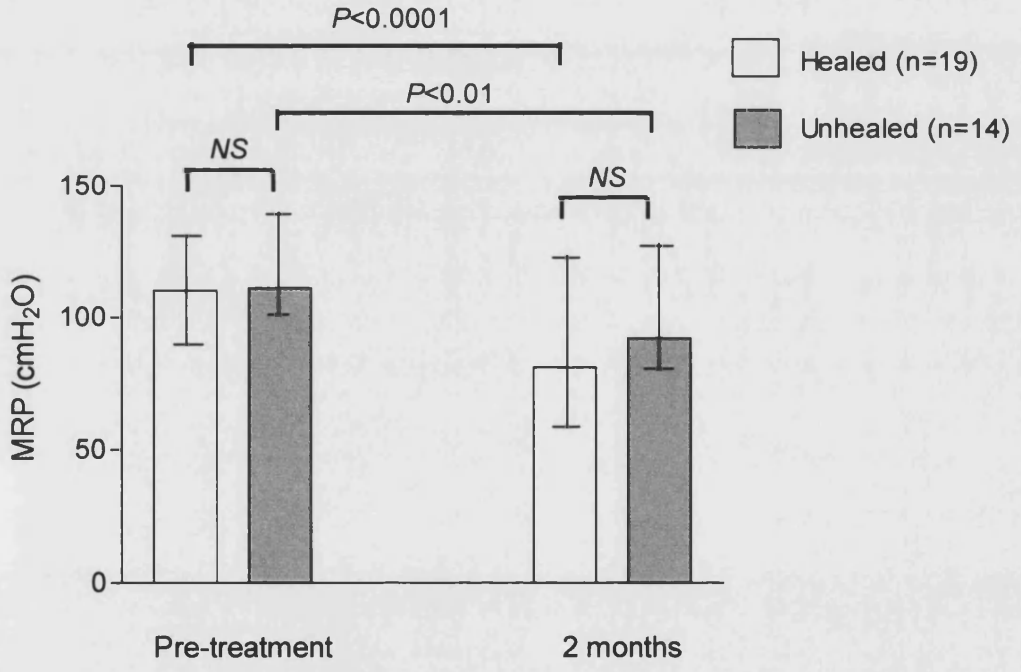


Figure 3.4. The median MRP in 19 patients healed and 14 patients who did not heal after 2 months treatment with 2% topical diltiazem. Interquartile ranges are displayed.

The MRP in those with anal spasm and those with sentinel piles is shown in *Appendices 6 and 7*. The MRP in those with anal spasm (19 of 33) was higher than those without [124 (93-227) v 99 (77-142) cmH_2O, *P<0.01*] *(fig.3.5.)*; the duration of symptoms in both groups was similar [4 (1-36) v 5 (2-30) months, *P*=NS]. Of 19 patients whose fissures healed 13 had anal spasm prior to treatment, in these patients the MRP was significantly reduced over 2 months by 5% [115 (93-195) v 109 (53-147) cmH_2O, *P*<0.005], while of 14 patients whose fissures did not heal 6 had anal spasm before treatment; in this group the reduction in MRP of 16% over 2 months was not significant [133 (93-227) v 112 (56-136) cmH_2O, *P*=NS]. The number of patients with fissures and anal spasm that healed and did not heal was not different (*Fischer's exact test*, *P*= 0.17).

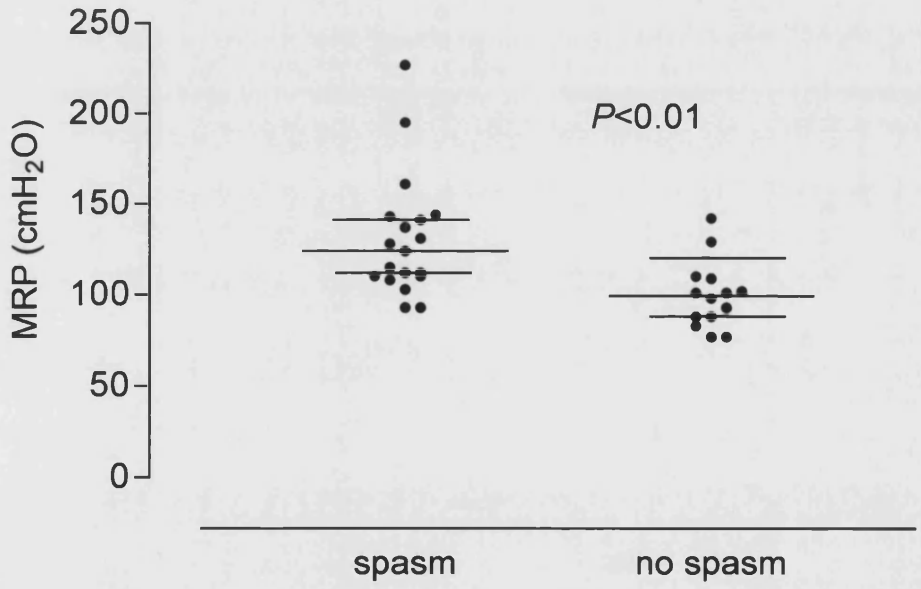


Figure 3.5. The median MRP in 19 patients with chronic anal fissures and anal spasm and 14 patients with fissures without anal spasm prior to treatment with topical 2% diltiazem. The horizontal lines represent the median values with interquartile ranges.

The MRP in those with sentinel piles (22 of 33) was higher than those without [120 (77-227) v 93 (77-144) cmH_2O, $P<0.005$] (*fig.3.6.*); the duration of symptoms in both groups was similar [6 (3-36) v 3 (1-12) months, P=NS]. Sentinel piles were present in 11 of 19 patients that healed, in whom the MRP was significantly reduced by 30% [115 (77-195) v 81 (53-136) cmH_2O, $P<0.001$] and in 11 of 14 patients that did not heal, in whom the MRP was also reduced significantly by 27% [128 (101-227) v 94 (56-136), $P<0.005$]. The MRP in patients with a sentinel pile with healed and unhealed fissures before treatment did not significantly differ [115 (77-195) v 128 (101-227) cmH_2O, P=0.54]; this was also the case comparing MRP after treatment [81 (53-136) v 94 (56-136), P=0.64]. The number of patients with fissures and sentinel piles that healed and did not heal was not different (*Fischer's exact test,* P=0.28).

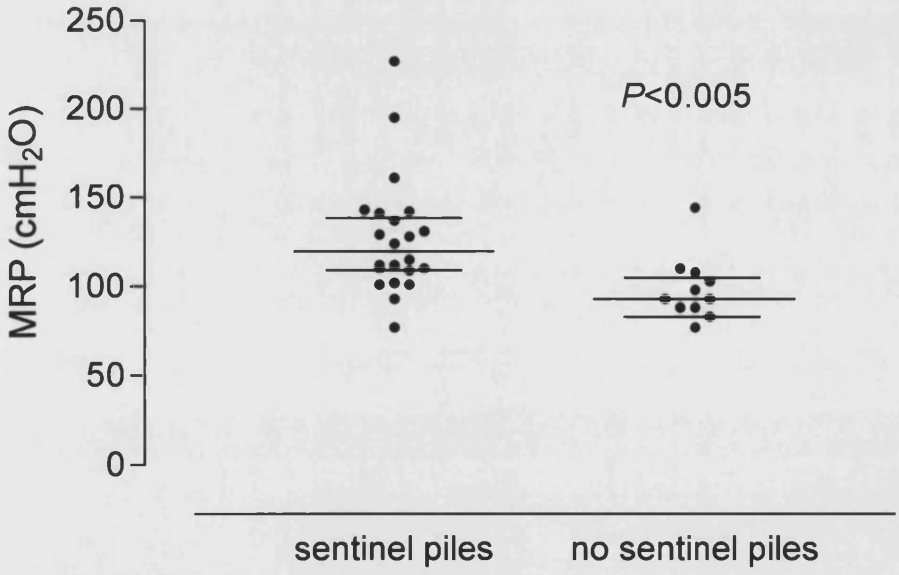


Figure 3.6. The median MRP in 22 patients with chronic anal fissures and sentinel piles and 11 patients with fissures without sentinel piles prior to treatment with topical 2% diltiazem. The horizontal lines represent the median values with interquartile ranges.

Assessment at 4 months

The results are shown in *Appendices 8 and 9.* Twelve patients whose fissures healed at 2 months remained healed. In this group the MRP was significantly higher than in controls [114 (88-195) v 88 (46-175) cmH_2O, *P*=0.02] and was reduced by 16 % at 2 months [114 (88-195) v 96 (53-147) cmH_2O, *P*=0.005], but at 4 months there was no change in the MRP [96 (53-147) v 113 (51-142) cmH_2O , *P*=0.19] and was no longer different from the pre-treatment level [114 (88-195) v 113 (51-142) cmH_2O, *P*=0.07] *(fig.3.7.)*. The MRP in the 12 patients prior to treatment was significantly higher than in controls [114 (88-195) v 88 (46-175) cmH_2O, *P*=0.02]. The MRP achieved at 2 [96 (53-147) cmH_2O] and 4 months [113 (51-142) cmH_2O] did not differ from controls [88 (46-175) cmH_2O, *P*=0.79 and 0.24 respectively].

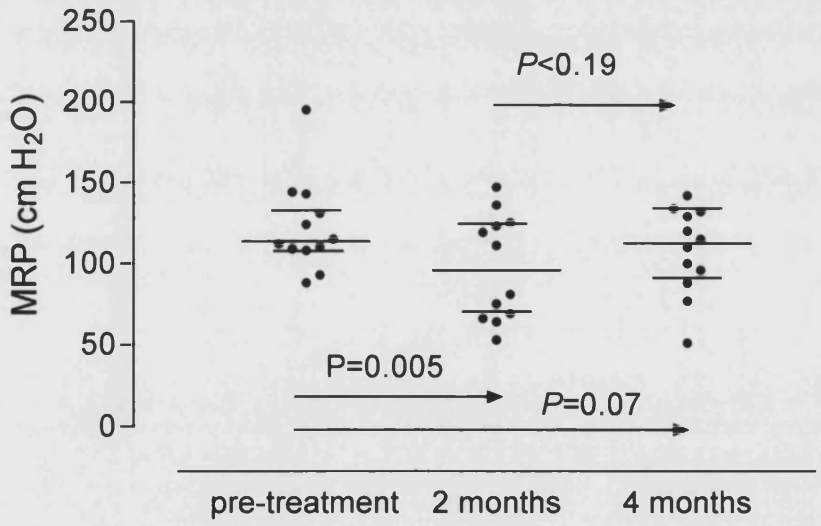


Figure 3.7. The median MRP in 12 patients with healed anal fissures that remained healed after a 2 month course of topical 2% diltiazem. The horizontal lines represent the median values with interquartile ranges.

In the other 8 patients with unhealed fissures after the initial course of treatment a reduction of 24% was not significant [106 (77-227) v 81 (56-136) cmH_2O, P=0.052] and at 4 months after a second course of treatment there was no significant change in the MRP from pre-treatment levels [106 (77-227) v 109 (47-135), P=0.29] *(fig.3.8.)*. However the fissures healed in 6 of 8 by 4 months; the MRP in this group was unchanged over the four months [102 (77-227) v 93 (79-136) v 109 (47-134) cmH_2O] and did not differ from controls [88 (46-175) cmH_2O]. There was no statistical difference between pre-treatment MRP in those 8 patients who required and 12 patients who did not require a second course of diltiazem [114 (88-195) v 106 (77-227) cmH_2O, P=0.92]. There was also no significant difference between the pre-treatment MRPs in the 8 patients and controls [106 (77-227) v 88 (46-175) cmH_2O, P=0.10].

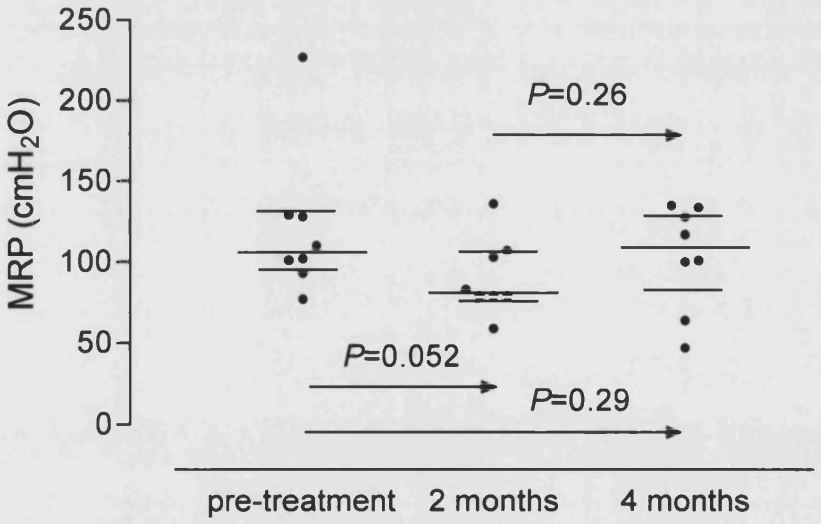


Figure 3.8. The median MRP in 8 patients with unhealed fissures at 2 months who required a second course of treatment with topical 2% diltiazem. The horizontal lines represent the median values with interquartile ranges.

Assessment at 6 months

The results are shown in *Appendix 10.* Of 14 patients with fissures that had healed by four months all remained healed, except one who after initially healing after 2 months recurred and one patient who had treatment for 4 months the fissure remained unhealed *(fig. 3.1.).* All patients were asymptomatic. The MRP in the 13 patients that remained healed is shown in *fig. 3.9.*; there was a significant reduction at 2 months but not at 4 months compared with pre-treatment values [110 (77-144) v 79 (53-147) v 108 (64-142) v 125 (53-161) cmH_2O]. At each stage from pre-treatment values to 6 months there was no difference from control MRP [88 (46-175) cmH_2O; P=0.11, P=0.67, P=0.21, P=0.09] respectively.

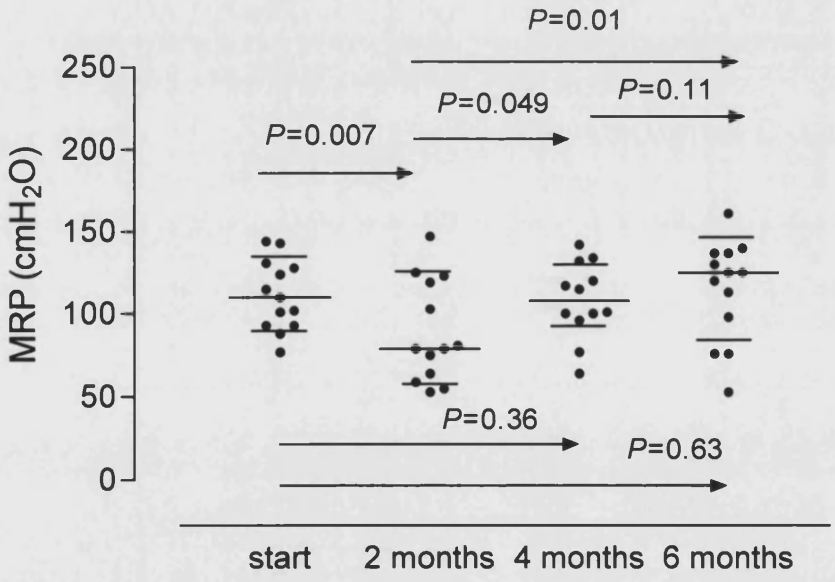


Figure 3.9. The median MRP in 13 patients with chronic anal fissures who had remained healed at 6 months after treatment with 2% topical diltiazem. The horizontal lines represent the median values with interquartile ranges.

3.4. DISCUSSION

While there have been several reports in the therapeutic effectiveness of diltiazem in chronic anal fissures few have discussed the manometric changes in the long term. Carapeti *et al.* (2000) and Jonas *et al.* (2001b) have included anal manometry in their reports with short follow up of 8 weeks, Knight *et al.* (2001) and Griffin *et al.* (2002) have follow up periods of 31 and 32 weeks without manometry. This study combined manometry during a six-month follow up that allowed analyses of the clinical response in relation to manometric changes.

Carapeti *et al.* (1999b) demonstrated diltiazem caused a sustained reduction of 28% in the MRP that lasted 3 to 5 hours. At present the published data suggests that the healing rates after one course of 8 weeks topical treatment with diltiazem have been 67% (Carapeti *et al.* 2000), 73% (Knight *et al.* 2001), 65% (Jonas *et al.* 2001b), 26% (Kocher *et al.* 2002), and 48% (DasGupta *et al.* 2002). In this study the healing rate of 19 (58%) of 33 is comparable.

The MRP in 33 patients was higher than healthy volunteers [110 (77-227) v 88 (46-175) cmH_2O] and this conforms with other reports [Nothmann and Schuster (1974), Farouk et al. (1994)]. Sub group analyses of those that healed and did not heal showed that 19 responders and 14 non responders had similarly higher [110 (77-195) v 111 (77-227) cmH_2O, *P*=NS] MRP than controls [88 (46-175) cmH_2O]. Carapeti *et al.* (2000) have also found no difference in pre-treatment MRP in responders and non responders [120

(85-156) v 136 (110-140) cmH_2O]. The reduction in MRP of 25% in the whole group of 33 patients after compares with the 24% reduction [127 (18) v 96 (17) cm H_2O, mean (sd)] after 8 weeks described by Carapeti *et al.* (2000) and Jonas *et al.* (2001). In the 19 that healed the MRP after 8 weeks reduced significantly by 26%, and in the 14 that did not heal the MRP also reduced significantly by 17%. In this study like Carapeti *et al.* (2000) the postreatment MRPs in the 19 responders and 14 non responders [81 (53-147) v 92 (56-136) cmH_2O, *P*=NS] were not different and were similar to the MRPs in controls. The reason for those patients with chronic anal fissures that failed to respond to therapy despite a reduction in MRPs similar to those fissures that healed and to a level within the range in healthy volunteers is unclear. One possible explanation is that treatment failed to achieve an anal canal pressure that allows adequate perfusion of the anoderm although Carapeti *et al.* (2000) failed to demonstrate an increase in anodermal blood flow with a reduced MRP with 2% topical diltiazem in 15 patients with fissures.

At 4 months 12 patients who healed at 2 months remained healed and whilst their MRP reduced during the treatment period it returned to pre-treatment values after cessation. There is no long-term manometric data in the literature to support this observation with 2% diltiazem. Of 8 patients that received a second course of 2% diltiazem although there was a trend there was no change in the MRP at any stage of assessment. However 6 healed by 4 months, and this cannot be explained within our current understanding of the pathophysiology of fissures. It can be hypothesised that either the local application of diltiazem relaxes the smooth muscles of the anodermal vessels, but this has not been corroborated with vascular flux studies; or the diltiazem acts as a placebo, as Altomare *et*

al. (2000) demonstrated healing in 29 (49.2%) of 59 patients that received placebo. In this series the healing rate at 4 months was 18 (90%) of 20, which includes the 12 that received one course and 6 that received a second course.

Evaluation at 6 months in 15 patients demonstrated that although all remained asymptomatic 13 (87%) healed. The MRP at 6 months had returned to pre-treatment levels [110 (77-144) v 125 (53-161)]. Although 1 (7%) of the 14 that healed by 4 months had recurred there is not enough data to reliably support this recurrence rate, though Griffin *et al.'s* (2002) analysis of patients treated with diltiazem showed that at 31 (median) months 7 (41%) of 17 described a recurrence of symptoms.

This "reversible" chemical sphincterotomy demonstrated in this study differs from lateral sphincterotomy in two respects. Firstly the reduction in MRP is not of the same magnitude, being approximately 50% with surgery [Boulos and Araujo (1984)] as compared to 16% (in this group). Secondly the effect is not sustained after cessation of treatment, as Chowcat *et al.* (1986) demonstrated that a reduction of about 50% in MRP was maintained 12 months after surgery.

Whilst manometry does not provide an indication of which patients at the start of therapy will heal; as differences in baseline pressure have no statistically significant effect on healing rates at 2 months (P=0.51), it does allow tentative conclusions to be made about the responses of patients with fissures to topical diltiazem. This study classifies chronic anal fissures into those with:

1: *High resting pressures that heal with a reduction of MRP.*

Demonstrated by 19 patients that healed with 2 months treatment in whom the MRP was significantly reduced by 26% [110 (77-195) v 81 (53-147) cm H_2O, *P<0.0001*] to a level similar to controls.

2: *High resting pressures that fail to heal despite a reduction in MRP.*

Demonstrated by 14 patients that did not heal with 2 months treatment in whom the MRP was significantly reduced by 17% [111 (77-227) v 92 (56-136) cmH_2O, *P<0.01*] to a level similar to controls.

3: *Normal resting pressures that heal although the MRP is unaltered with treatment.*

Demonstrated by the 6 patients who received 4 months treatment and healed, where the MRP was not reduced [102 (77-227) v 109 (47-134) cmH_2O, *P*=NS].

Few investigators have examined the relationship of the physical signs associated with fissures and their healing. Lock and Thompson (1977) noted that the presence of a sentinel tag or fibrous anal polyp were significant features of chronicity and of conservative treatment. Gough and Lewis (1983) demonstrated that a sentinel tag was present in 18 (55%) of 33 patients whose posterior fissures healed on conservative therapy compared with 23 (50%) of 46 whose fissure persisted despite treatment. Analyses of their results demonstrated that the presence of sentinel piles was not related to healing (*Fischer's exact test, P*= 0.82). Pitt *et al.* (2001a) in a Cox model multivariate analysis of 7 factors in 64 patients to determine significant factors related to healing demonstrated that a history of a fissure exceeding 6 months and the presence of a sentinel

pile were associated with failure of the fissure to heal. The presence of a sentinel pile also predicted higher recurrence rate. Analysis of the data in this study indicates that the duration of symptoms did not relate to the presence of anal spasm or sentinel pile, although those with spasm had a higher MRP. Important differences between the work in this chapter and Pitt *et al.*'s (2001a) study was that the latter study determined factors until 12 weeks with treatment, and 0.2% GTN was used instead of 2% diltiazem. Furthermore in Pitt et *al.*'s study, where GTN was used, the recurrence rate of 46% was within 32 weeks, and our study, where diltiazem was used, extended to 24 weeks where 1 (7%) of 14 patients that had healed, recurred.

Diltiazem is an effective agent used to heal chronic anal fissures by causing a reduction in MRP in most patients. The IAS of patients with fissures that do not heal may express an abnormal physiological behaviour that is yet undefined. Anal canal manometry differentiates those with higher MRPs from those with normal resting pressures, in whom a reduction with topical agents may not be demonstrated. It is not yet possible to determine accurate prognostic factors for healing.

CHAPTER FOUR

NOVEL DELIVERY OF BOTULINUM TOXIN FOR TREATMENT OF ANAL FISSURES

4.1 INTRODUCTION

Botulinum toxin is an endopeptidase which blocks acetylcholine release at the neuromuscular junction of alpha motor neurones, at gamma neurones in muscle spindles and in all parasympathetic and cholinergic postganglionic sympathetic neurones. It is only recently that a study by Jones *et al.* (2002a) described the mechanism of action of botulinum toxin on the IAS. The electrically field stimulated responses (EFS) to isolated porcine IAS tissue were compared before and after incubation with botulinum toxin. It was found that treatment with this agent increased myogenic tone by 38%. Whilst the EFS-induced relaxations were unaffected, the EFS-induced contractions were significantly reduced. It was concluded that the blockade of sympathetic output by botulinum toxin probably outweighed its effect on increased myogenic tone and botulinum toxin blocked sympathetic nerves distal to their ganglia, by reducing noradrenaline release at the neuromuscular junction, but had no effect on nitregic transmission.

Of the different forms of botulinum toxin produced by the bacterium *Clostridium botulinum*, the most commonly used in a therapeutic role is the type A. It has now been purified to homogeneity and has been employed in the treatment of various neuromuscular disorders. Wright (1955) examined its potency, and demonstrated a dose of 0.003 μg of botulinum toxin, administered parenterally, would be lethal to an adult human. Although classic absorption, distribution, biotransformation and elimination studies on the active substance have not been performed in the human, it is believed that little systemic distribution of therapeutic doses of Botox® occurs (Allergan, personal communication). There are two commercially available forms of

botulinum toxin type A, Botox® (Allergan, Irvine, Ca, USA) and Dysport® (Ipsen, Dublin, Ireland). One unit of these preparations has the same LD_{50} but has different biological activity: 3-5 units of Dysport® correspond to 1 unit of Botox®. There are two reports on the use of Dysport® (see *table 4.1*), the dosages used range from 20 to 50 units. Another form of botulinum toxin, type B (Neurobloc, Elan Corporation, Dublin, Ireland) has been recently introduced. There have been 14 reports of botulinum toxin injection into the IAS for the treatment of anal fissures (*table 4.2.*), one report by Espi *et al.* (2001) was a long term study. There have been 10 reports of injection into the EAS including two reports by Jost and Schimrigk that described the complications and effects on the EAS (*table 4.1.*).

The IAS and EAS maintain the MRP of which 75-85 % is contributed by the IAS (Frenckner and Euler, 1975, Schweiger, 1979). Botulinum toxin type A, Botox® in different doses injected into the IAS induces reduction in MRP that varied from 16 to 34 % that is maintained for several weeks and is transient; Borodie *et al.* (1974) indicated that the paralysing effects last for approximately 3 months. There is also a reduction in the voluntary anal canal pressure, ranging from 0 to 21 %. Madalinski *et al.* (1999) suggested this apparent reduction of EAS tone may be directly related to injection, as botulinum toxin diffuses from the injection point up to a distance of 35-40mm. The manometric data for injections into the EAS have not been adequately reported but Jost and Schimrigk (1994) stated that, at completion of treatment, the sphincter tone was within normal limits. In Jost's (1997b) later analysis of 100 patients at 6 months after injection he demonstrated healing and a normal sphincter tone in 79 patients.

Table 4.1. Effect of injection of botulinum toxin type A injected into the external anal sphincter in patients with anal fissures

AUTHORS	DOSE (units)	PATIENTS (number)	REVIEW (weeks)	HEALING RATE (%)	MAXIMUM REDUCTION IN RESTING PRESSURE (%)	MAXIMUM REDUCTION IN VOLUNTARY PRESSURE (%)
Jost&Schimrigk (1993)	2.5	1	12	100	No data	No data
Jost&Schimrigk (1994)	2.5	12	12	83	No data	No data
Jost&Schimrigk (1995)	5	26	12	81	No data	No data
Jost (1997a)	2.5 - 5	100	12	82	No data	No data
Jost & Schrank (1999a) *	20 40	50	12	76 (20U) 80 (40U)	No data	No data
Jost & Schrank (1999b)	5 10	20 30	12	70 (5U) 63 (10U)	No data	No data
Jost (2001) #	200	10	4	70	No data	No data
Thompson *et al* (2002)*	50	26	12 -not complete	81	No data	No data

- * Dysport used for injection
- # Botulinum toxin type B used for injection

Table 4.2. Effect of injection of botulinum toxin type A injected into the internal anal sphincter in patients with anal fissures

AUTHORS	DOSE (units)	PATIENTS (number)	REVIEW (weeks)	HEALING RATE (%)	MAXIMUM REDUCTION IN RESTING PRESSURE (%)	MAXIMUM REDUCTION IN VOLUNTARY PRESSURE (%)
Giu *et al.* (1994)	15	10	8	70	25	21
Mason *et al.* (1996)	0.125-1 ng	5	12	60	16	No data
Espi *et al.* (1998)	10 - 15	36	24	65 (10U) 81 (15U)	No data	No data
Maria *et al.* (1998a)	20	15	4 & 8	53 (4 wks) 73 (8 wks)	28	44
Maria *et al.* (1998b)	15 - 20	57	8	44 (15U) 67 (20U)	29 (15U) 28 (20U)	17 (15U) 13 (20U)
Brisinda *et al.* (1999)	20	25	8	96	29	6
Minguez *et al.* (1999)	10 - 21	69	24	83 (10U) 78 (15U) 90 (21U)	5 (10U) 13 (15U) 16 (21U)	17 (10U) 17 (15U) 35 (15U)
Fernandez *et al.* (1999)	80	76	12	67	No data	No data
Maria *et al.* (2000)	20	50	8	74	32	5
Lysy *et al.* (2001)	20 ± ISDN	30	12	73 (ISDN) 66	24 (ISDN) 21	4.8 (ISDN) 4.4
Madalinski *et al* (2001)	50	13	No data	54	No data	No data
Gecim (2001)	5	27	6	80	No data	No data
Brisinda *et al.* (2002)	20 - 50	150	8	89 (20±30U) 96 (30±50U)	30 (20±30U) 34 (30±50U)	0 (20±30U) 8 (30±50U)
Katory *et al.* (2002)	50	20	6	80	No data	No data

There are few studies that describe complications from injection and no studies to indicate patients' attitude to this form of treatment. Maria *et al.* (2000) did not observe any complications or side effects in 50 patients they treated. Madalinski *et al.* (2002) specifically addressed the side effects of botulinum toxin injection for benign anal disorders in 105 patients with anal fissures and 34 patients with functional outlet obstruction. Those with fissures complained of incontinence of flatus (n=9), incontinence to faeces (n=5), anal haematoma (n=5), flu-like syndrome (n=3), acute inflammation of external varices (n=2), epdidymitis (n=1) and haemorrhoidal prolapse (n=1). Patients with anismus suffered from intertrigo (n=1), and prolonged pain up to 4 days (n=4). However none experienced life threatening side effects. Madalinski *et al.* (2001) injected a higher dose of 100 units into the IAS of a male patient with an unhealed fissure with a successful outcome and without complication. While tachyphylaxis has not been addressed specifically repeated treatment has been attempted successfully without side effects. Jost and Schrank (1999b) repeated an injection of 5 units in 20 patients with recurrent fissures, 14 (70%) healed by 3 months. In a second group, comprising of 30 patients with initial therapeutic failure of botulinum toxin with 5 units, when administered 10 further units, showed that 19 (63%) 30 healed at 3 months.

There have been several studies on botulinum toxin injection treatment for chronic anal fissures but few have reported on the recurrence rate (*table 4.3.)*. Whilst in several series with a mean follow up of at least one year where no recurrences were demonstrated; Jost and Schrank (1997b, 1999a) had a recurrence of 8% after injection into the EAS at 3 and 6 months and Espi *et al.* (2001) had a much higher recurrence rate of 42% after injection into the IAS at a median follow up of 38 months.

Table 4.3. Recurrences after injection of botulinum toxin in the treatment of anal fissures

AUTHORS	DOSE (units)	FOLLOW UP (months)	RECURRENCE RATE (%)
Maria *et al.* (1998b)	15 - 20	25 ± 6	No recurrences
Maria *et al.* (2000)	20	18 ±7	No recurrences
		20 ±4	No recurrences
Espi *et al.* (2001)	No data	38 (26-45)	42
Brisinda *et al.* (2002)	20 ± 30	21 ± 5	No recurrences
	30 ± 50	23 ± 4	No recurrences
Jost & Schimrigk (1995)	5	12	No recurrences
Jost (1997b)	2.5 - 5	6	8
Jost & Schrank (1999a) *	20 -40	3	8

- * Dysport used for injection

Jost (2001) examined the efficacy of another form of botulinum toxin, type B (Neurobloc® , Elan Corporation, Dublin, Ireland) in the treatment of 10 patients with anal fissures and reported a healing rate of 70% after one month. The only complaint was of burning after injection in 4 patients, probably related to the lower pH of this preparation. There have been no other reports on this product.

Reluctance for clinicians to use botulinum toxin to treat anal fissures may be related to complications of injection. Jost *et al.* (1995) reported perianal thrombosis in 5 of 26 female patients treated with botulinum toxin injected into the EAS, whereas two of ten patients injected into the intersphincteric groove by Tilney *et al.* (2001) returned within a week of injection with perianal haematomas.

In order to avoid distress to patients and improve compliance, perhaps also to minimise the risk of potential complications of direct toxin injection the use of the "J-Tip® Needle-less Injection System" (National Medical Products Inc., Irvine, California, USA) used by insulin-dependent diabetics seemed an attractive option *(fig. 4.1.)*. The physical characteristics include an ejection pressure of approximately 3,000 psi, a delivery time of 0.2 s, and a penetration depth of 5-8 mm, which 'fans out' to cover an area of 1-1.5 cm [Keshtgar and Barker 1999].

The aim of this study was (i) to carry out preliminary work to determine the optimal site and method of application of the needle-less syringe to ensure that the injected fluid reached the IAS in a porcine anal canal model and (ii) to investigate the efficacy of injection of the J-Tip® needle-less injection system in the treatment of chronic anal fissures.

The **J-Tip®** needle-less syringe system has three components *(fig. 4.1.)*:

1. **The J-Tip Needleless Injector** is a single use, pre-sterilized, disposable unit which is similar to the customary syringe in the delivery of medications and contains it's own power source (CO2) to deliver the medication through the skin into the subcutaneous tissue.

2. **The J-Tip Adapter** is attached to the botulinum toxin bottle enabling the user fill the J-Tip Transporter with the appropriate volume dosage.

3. **The J-Tip Transporter** allows medication to be taken from the botulinum toxin bottle and to be filled into the J-Tip injectors.

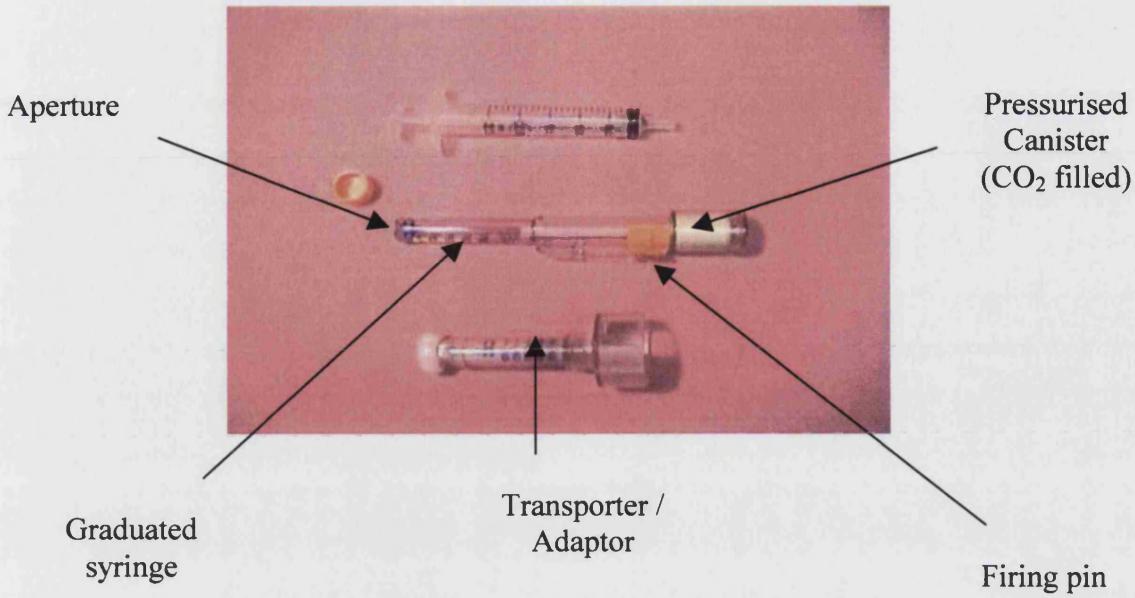


Figure 4.1. The J-Tip® needle-less syringe

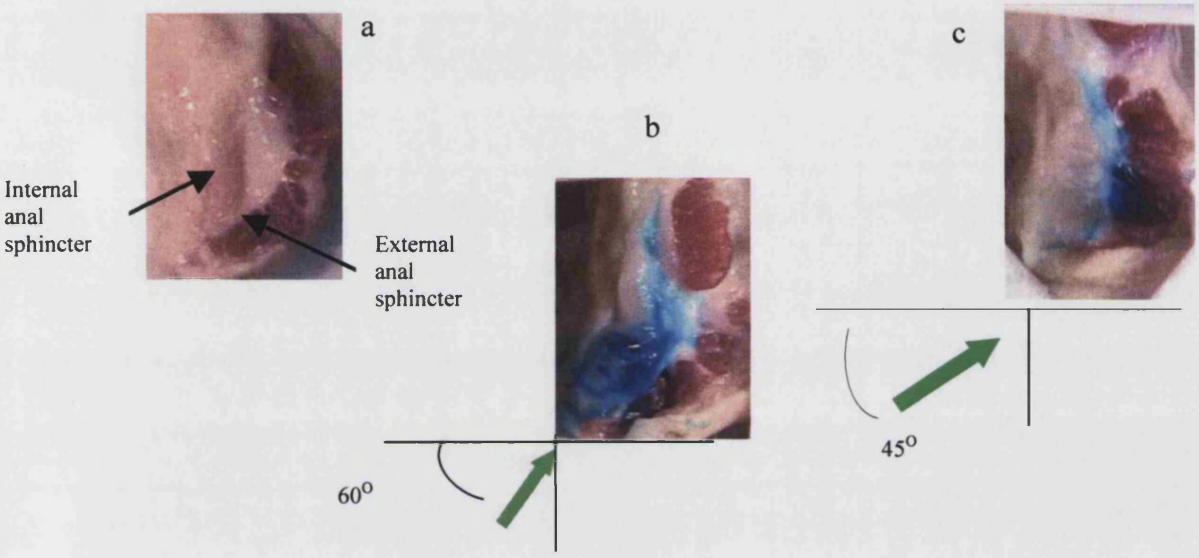
4.2. PRE-CLINICAL STUDY

Methods and patients

Twenty-four specimens of porcine anal canals were retrieved from the abattoir and transported to the laboratory within 3 hours in modified Kreb's solution. Botox® has a molecular weight of 150 kd and as the colloid eloHAES® (Fresnius) has a similar average molecular weight of 200 kd it was selected to be the carrier for the methylene blue. In order to visualise the distribution of the injected agent after penetration the eloHAES® was mixed with methylene blue (molecular weight of 373.9 d) (David Bull Laboratories, UK). Injections of 0.25ml volume were delivered at the anal verge in six specimens at each angle, ranging from 45° to 90°, in 15° increments from the horizontal plane of the skin. The sphincter complex was then dissected and the most

optimal angle that produced consistent penetration into the IAS, as judged visually by the investigator, was selected for the clinical study *(fig.4.2.)*.

Figure 4.2. **a) The normal anatomy of the porcine anal sphincter complex.**
b) Injection at 60° to the horizontal plane
c) Injection at 45° to the horizontal plane



Results

The angle that caused consistent infiltration of the IAS with all six specimens was 60° to the horizontal plane (*Fig. 4.2.b.*). The other angles of injection 45°, 75°, and 90° failed to penetrate the IAS adequately with injections. With 75° and 90° injection angles the submucosal plane was predominantly penetrated with methylene blue and with 45 ° the intersphincteric plane was penetrated (*Fig. 4.2.c.*).

4.3. CLINICAL STUDY

Methods and patients

Local Ethical Committee approval for the study was obtained for this study. Ten consecutive patients; 5 males, median age 40 (range 22-71) years; with chronic anal fissures not previously treated were recruited to have two injections of 25 units of Botox® injected either side of the anal fissure at an angle of 60° to the horizontal plane of the anal verge in an outpatient setting without anaesthetic. The exclusion criteria were as follows:

- Aged <18 years or > 75 years
- Inflammatory bowel disease
- Perianal sepsis or fistula
- Diabetes, glaucoma or neuromuscular disorders
- Immunosuppresion
- Pregnancy and breast feeding
- Previous significant anal trauma or surgery with residual severe scarring
- Suspicion of infective or neoplastic cause for the fissure

Patient assessment included clinical examination, a graduated visual analogue pain score, an incontinence score [Vaizey *et al.* (1999)] *(fig. 4.3.),* and anal manometry. The measurements were repeated at 1, 4, 8 and at 12 weeks after treatment, the fissure examined and was regarded as healed when it was not tender on palpation and the site of the fissure had re-epithelialised. Statistical analyses were conducted with paired t tests after Gaussian distribution of the differences in measurements had been confirmed.

Analogue pain score

0-1-2-3-4-5-6-7-8-10

0 = no pain
10 = the worst pain imaginable

Incontinence score [Vaizey et al. (1999)]

	Never	Rarely	Sometimes	Weekly	Daily
Incontinence for solid stool	0	1	2	3	4
Incontinence for liquid stool	0	1	2	3	4
Incontinence for gas	0	1	2	3	4
Alteration in lifestyle	0	1	2	3	4

	No	Yes
Need to wear a pad or plug	0	2
Taking constipating medicines	0	2
Lack of ability to defer defaecation for 15 min	0	4

Never	No episodes in the past 4 weeks
Rarely	1 episode in the past 4 weeks
Sometimes	> 1 episode a week in the past 4 weeks < 1 a day
Daily	1 or more episodes a day

Add one score from each row:
Minimum score = 0 = perfect continence
Maximum score = 24 = totally incontinent

Figure 4.3. Analogue pain score and Incontinence score

Results

Patients did complain of discomfort after the injection but were fully mobile after the procedure. There were no complications except for one patient who developed a 3mm haematoma at the injection site. The results are shown in *Appendices 11, 12, 13* and summarised in *table 4.4*. At 12 weeks 6 patients had complete resolution and 2 marked improvement of pain; 5 of whom the fissures healed. One patient remained symptomatic and withdrew from the study at 8 weeks and another was symptomatic with an unhealed fissure at 12 weeks.

Time post injection (weeks)	Pain score (0-10) median (range)	Incontinence score (0-24) median (range)	MRP (cmH_2O) median (range)
Start	6 (3-8)	3.5 (0-9)	99 (71-185)
1	4 (1-8)	4 (0-10)	81 (37-166)
4	3 (0-8)	4 (0-14)	93 (70-109)
8	2.5 (0-8)	3 (0-15)	86 (51-110)
12	3 (0-7)	0 (0-15)	101 (74-143)

Table 4.4. The median pain scores, incontinence scores and MRP at 1, 4, 8 and 12 weeks post injection of 50 units of Botox® in 10 patients with chronic anal fissures.

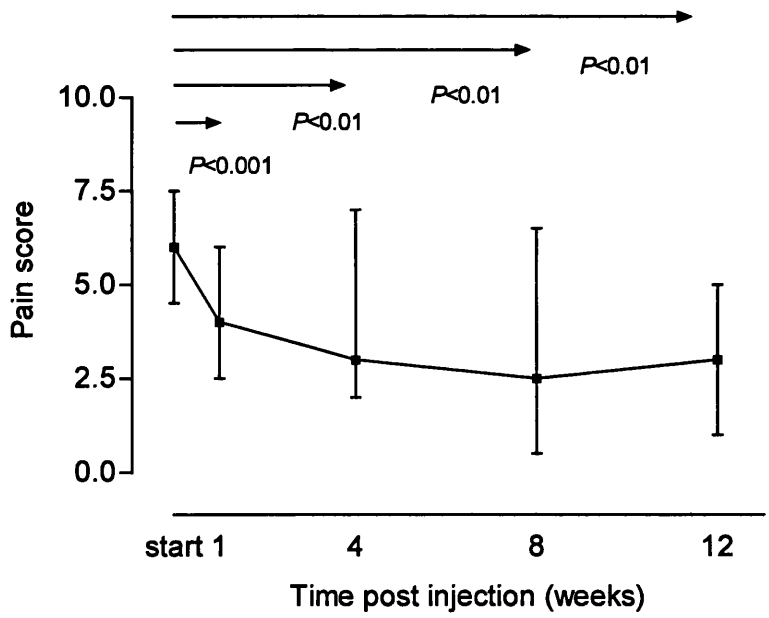


Figure 4.4. The median pain scores (0-10) over a 12 week period after injection of 50 units of Botox® in 10 patients with chronic anal fissures. The error bars indicate interquartile ranges.

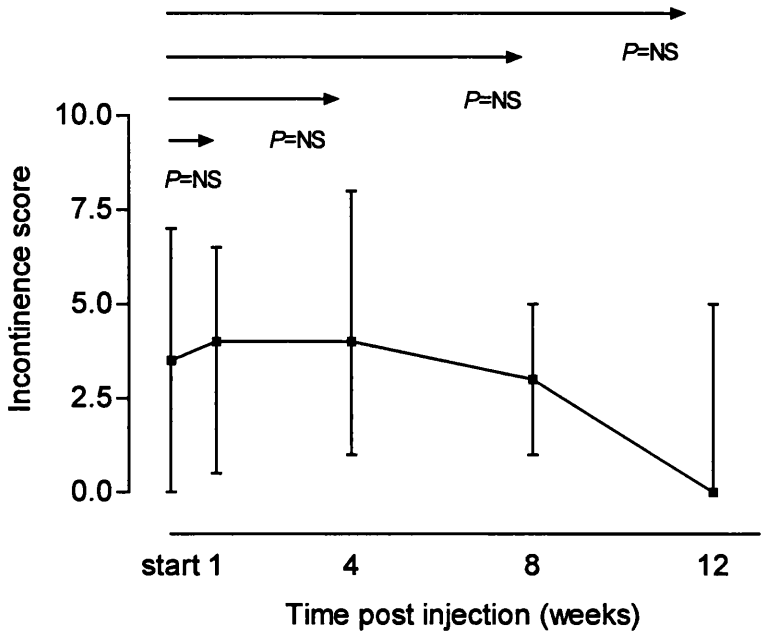


Figure 4.5. The median incontinence scores (0-24) over a 12 week period after injection of 50 units of Botox® in 10 patients with chronic anal fissures. The error bars indicate interquartile ranges.

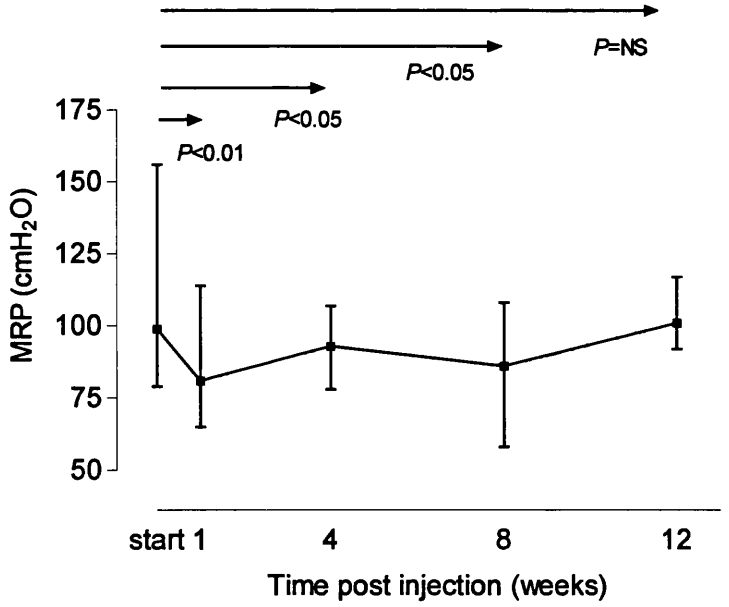


Figure 4.6. The median MRP over a 12 week period after injection of 50 units of Botox® in 10 patients with chronic anal fissures. The error bars indicate interquartile ranges.

At all stages the pain score was significantly decreased, this was reflected in the 8 patients who had complete resolution (n=6) or improvement (n=2) of pain (*fig. 4.4.*). Although there were no statistically significant changes in incontinence throughout the study period one patient described a single episode of urge incontinence at 1-week post injection and another patient described a single episode of passive incontinence at 8 weeks (*fig. 4.5.*). The reduction of median MRP after 1, 4 and 8 weeks was 18, 6, and 13% respectively (*fig. 4.6.*). These reductions maintained significance until 8 weeks, but at 12 weeks the MRP did not differ from pre-treatment values. In the patient with passive incontinence the MRP had reduced maximally at this stage from 92 cmH_2O (start) to 66 cmH_2O (8 weeks), reflecting a decrease in the MRP of 28%. In the patient with urge incontinence the MRP had reduced maximally from the start from 91 cmH_2O (start) to 85 cmH_2O (1 week), reflecting a decrease in the MRP of 7%. The median MRP for the group had by comparison decreased from 99 cmH_2O (start) to 81 cmH_2O and then to 86 cmH_2O (8 weeks). Thus the overall healing rate was 5 (50%) of 10 studied.

4.4. DISCUSSION

The experimental studies have demonstrated the need for precise problems relating to the unique anatomy of the anal canal and the necessity to inject into the correct muscle structures. However as diffusion of the drug is expected over time it would be interesting to note the reduction in voluntary squeeze pressure, a manifestation of action on the EAS, which was outside the preliminary aim of this study. A similar large dose of Botox® is safe to be used as Madalinski *et al.* (2001) had used a 100 unit

dose to treat a patient with a non-healing fissure; also Fernandez-Lopez *et al.* (1999) had used 80 units with a 67% healing rate in 76 patients. It is unsure how much of the toxin molecule would be denatured as a result of the mode of delivery, however the evidence of effect on MRP did demonstrate that some of the toxin had acted until three months, which is keeping with duration of action as described by Borodie *et al.* (1974). We were unable to comment on its loss of potency. The maximal reduction in the median MRP of 18% in this study was demonstrated at one week; this compares favourably with the range of reductions outlined in *table 4.2.*, though comparative reductions of 16% were described by Mason *et al.* (1996) using 0.125-1 ng of toxin and Minguez *et al.* (1999) using 21 units of toxin. The healing rate of 5 (50%) of 10 in this study is lower than expected; this is surprising as a significant reduction of the MRP was demonstrated up to 8 weeks post injection. It has been shown that though one is able to target the injected solution one cannot determine whether the full dose is delivered; this may explain the poor healing rate. Another reason for the poor healing rate may be related to the magnitude of the reduction, as it was 18% after one week, and may not have been sufficient to allow perfusion of the anoderm to allow healing. In Brisinda *et al's* (2002) study healing rates of 89% and 96% were demonstrated after MRP reductions of 30% and 34% respectively. Dose ranging studies with botulinum toxin with the needless syringe would answer this and using higher doses could result in improved healing rates. In Madalinski *et al's* (2002) study of complications of direct toxin injection 5 (4.7%) of 105 patients with anal fissures developed haematomas, compared with 1 patient with the needle-less device. It is not possible to equate the initial discomfort of the needle-less device with that of direct injection (as no quantitative assessment of discomfort of the direct injection method has been made), but patients seem to be unperturbed by the second

firing of the needle-less syringe. The dose selected did not cause a significant disturbance in anal continence in 8 patients. Incontinence would be a reflection of the reduction in the resting and/or voluntary squeeze pressures, this is more important for botulinum toxin as it has an effect on both the smooth muscle of the IAS and the striated muscle of the EAS. Recurrences were not addressed by this study as the follow up period of 12 weeks was too short.

This study has demonstrated the feasibility of this mode of delivery of botulinum toxin in the treatment of chronic anal fissures. It has limitations as shown by the low healing rate, but appears to be safe and patients are able to tolerate the mode of delivery. Dose-response studies with higher number of patients with differing doses would answer unresolved issues highlighted.

CHAPTER FIVE

SYMPATHETIC MODULATION OF THE INTERNAL ANAL SPHINCTER

5.1. INTRODUCTION

Current treatments that induce relaxation of the IAS act by promoting the delivery of nitric oxide locally (glyceryl trinitrate, isosorbide dinitrate), by inhibiting the transfer of calcium across membrane channels (diltiazem, nifedipine) or by modulating the sympathetic effect (botulinum toxin). The IAS tone is partly maintained by continuous sympathetic input, mediated through excitatory α_1 adrenoceptors and inhibitory β_2 adrenoceptors. These ultimately change the intracellular homeostatic mechanisms that either promote uptake or release of calcium from the sarcoplasmic reticulum. Stimulation of the α_1 adrenoceptors increases intracellular calcium via inositol-1'4'5'-triphosphate (IP_3), whilst stimulation of β_2 adrenoceptors reduces intracellular calcium via cylic adenine-3'5'-monophosphate (cAMP).

Ojo-Aromokudo *et al.* (1998) studying the effects of salbutamol, a β_2 receptor agonist, with indoramin, an α receptor antagonist in volunteers and patients with chronic fissures noted that whilst there was no apparent difference in the α-adrenoceptors in the IAS of patients with anal fissures compared with controls, as measured by the comparative reduction in anal canal pressure after the administration of indoramin, the β-adrenoceptors were probably up-regulated in patients with anal fissures, as the reduction in anal canal pressure was greater in fissure patients than in controls. The up-regulated β-adrenoceptors have also been demonstrated by *in vitro* studies by Regadas *et al.* (1993) where supersensitivity to relaxation by isoproterenol, a β-adrenoceptor agonist, was demonstrated in IAS strips removed from patients undergoing lateral sphincterotomy, as compared with IAS strips removed from patients with third-degree haemorrhoids and normal anal canal pressures.

Pitt *et al.* (2000) reported the effect of a single 20mg oral dose of indoramin, α_1 adrenoceptor antagonist on MRP in 7 patients with chronic anal fissures, mean age 38 (range 24-48) years and 6 healthy volunteers, mean age 52.2 (range 30-71) years. Anal manometry was performed using a water-filled microballoon by the station pull-through method at 1 cm intervals, with equilibration of the pressure before a reading was taken. In the patient group the mean MRP was reduced significantly from 106.9 to 68.6 cmH_2O, a 35.8% reduction, at 1 hour. In volunteers the MRP was also significantly reduced from 84.2 to 52.2 cmH_2O, a 39.9% reduction. These significant reductions were maintained at 2 and 3 hours in both groups. No adverse cardiovascular or other effects were experienced by any subject in the study. The MRP in both controls and patients with anal fissures was measured at 1 to 2 cm from the anal verge; this profile was not altered by indoramin. This initial study prompted further investigation into indoramin as a therapeutic agent to treat chronic anal fissures.

Pitt *et al.* (2001b) then conducted a double-blind randomised placebo-controlled trial of oral indoramin to treat chronic anal fissure in 23 patients; 14, mean age 37 years, received 20mg oral indoramin; and 9, mean age 39 years received placebo. Seven patients in the indoramin group and two patients in the placebo group withdrew due to side effects within the first two weeks of treatment. The side effects included fatigue, dizziness, headache, dry mouth, nasal congestion, and retrograde ejaculation; and overall there were more side effects in the indoramin group. Pain was reduced in the placebo group but not in the indoramin group. At six weeks only 1 (7%) fissure healed in only 1 (7%) compared with 2 (22%) in the placebo group. The fissure that

healed with indoramin recurred at 3 months. On the basis of this disappointing result the investigators felt it was unethical to continue with the study due to ethical reasons.

The aim of the study was (i) to validate the effects that manipulation of IAS tone with indoramin, an α_1 adrenoceptor antagonist, and salbutamol, a β_2 adrenoceptor agonist have on IAS tone and (ii) to examine the pharmacological potential of topical formulations of these agents for the treatment of anal fissures.

5.2. EFFECT OF ORAL INDORAMIN AND ORAL SALBUTAMOL ON ANAL CANAL PRESSURE.

5.2.1. PATIENTS AND METHODS

There were 10 patients (median age 35.5, range 28-65 years, 6 females) with chronic anal fissures and 10 healthy volunteers (median age 28, range 21-38 years, 5 females) that were studied after local ethical committee approval was obtained. The MRP, pulse and blood pressure were measured before a single oral dose of 20mg indoramin or 4mg salbutamol in a cross over manner. Both the investigator and the participants were blinded as to the nature of the drug administered. The MRP, pulse and blood pressure were measured hourly post ingestion of the drugs until 3 hours; noticeable side effects were also recorded. A minimum "washout period" of 24 hours before the next drug could be given was observed. This was considered a sufficient period as the elimination half lives of indoramin and salbutamol are 5 [Pierce, 1990, Volans *et al.* (1982)] and 1-2 hours [Janson, 1991] respectively. Whilst 20mg indoramin is a dose

that is safely used to treat hypertensive patients, 4mg oral salbutamol is a considerably higher dose than that inhaled (200-400μg) for the treatment of asthma. However the agents and the doses were selected as they were those employed in the studies by Pitt *et al.* (2000) and Ojo-Aromokudo *et al.* (1998), and it was felt that employing the same doses were essential if any meaningful comparisons from this study were to be made. The differences within each dose were calculated with the paired t test after testing differences in MRP for normality.

5.2.2. RESULTS

The data are shown in *Appendices 14 to 19*, and in *table 5.1.* The hourly effect up to three hours of indoramin and salbutamol on the MRP in volunteers and patients with chronic anal fissures are shown. Also shown are the % reductions of the median MRP at each hour. At the start of the study patients with fissures had a significantly higher MRP than volunteers [131 (94-227) v 88 (46-175) cmH_2O, median (range), $P < 0.0001$] (*fig.5.1.*). In all 4 groups the MRP reduced significantly after one hour and this reduction was observed over the 3 hours studied (*table 5.1, figs 5.2-5.5.*). The reductions of the median MRP at 1 hour for indoramin in the patient group was 32.6 % and in the volunteer group was 25.4%; and there was no difference in the magnitudes of reduction of the MRP [52 (18-69) v 17 (4-121) cmH_2O, median (range), *P*=NS]. The reduction of the median MRP at 1 hour for salbutamol in the patient group was 29.6% and in the volunteer group was 30.4 %; again there was no difference in the magnitudes of reduction of the MRP [20 (7-81) v 25 (-1-105) cmH_2O, median (range), *P*=NS]. Whilst in 3 of the groups there was no further reduction in the MRP from 1 to 3 hours in the 10 volunteers that had received

indoramin there was a further significant reduction in the MRP from 2 to 3 hours [55 (46-114) v 53 (33-112), median (range), $P < 0.05$] (*fig. 5.2.*).

There was no difference in the pulse rate in the subjects that had received indoramin (*fig. 5.6.*). There was a transient significant increase in the pulse rate at 1 hour in volunteers and at 2 hours in patients that had received salbutamol (*fig. 5.7.*). There were no differences in systolic blood pressure in any group during the study period (*figs. 5.8. & 5.9.*). Four volunteers and five patients experienced tremors after salbutamol, three patients complained of light-headedness with indoramin, and none of the volunteers had any side effects with indoramin.

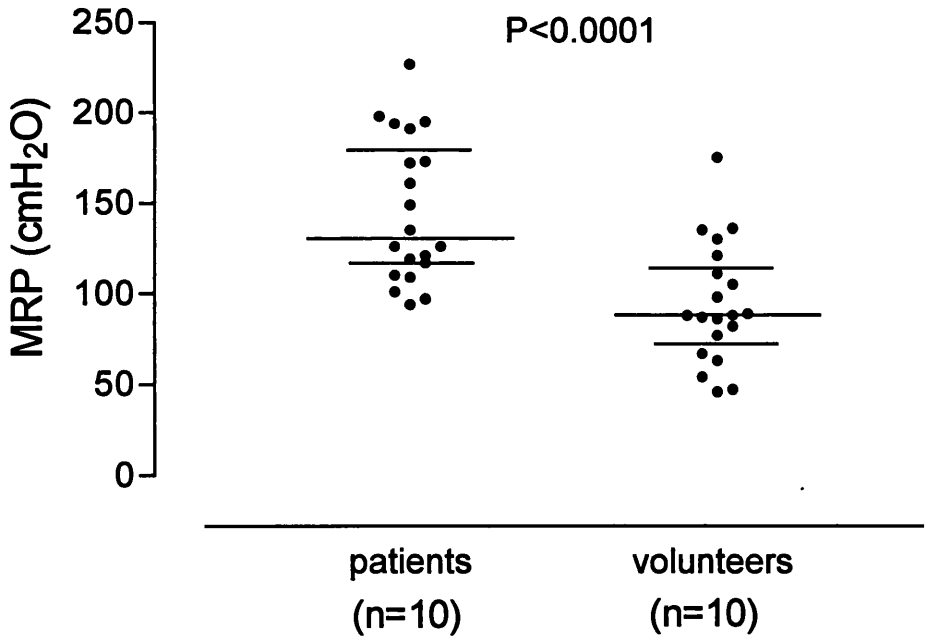


Figure 5.1. The median maximum resting anal canal pressure (MRP) in 10 patients with chronic anal fissures as compared with 10 healthy volunteers. The horizontal lines represent the median values with interquartile ranges

Table 5.1. The effect of 20mg oral indoramin and 4mg oral salbutamol on the maximum resting anal canal pressure

MRP (cmH_2O) at start (0), and 1, 2 and 3 hours after ingestion of drug		PATIENTS WITH ANAL FISSURES		HEALTHY VOLUNTEERS	
		INDORAMIN	**SALBUTAMOL**	**INDORAMIN**	**SALBUTAMOL**
O	Mean (S.E.)	147.0 (14.1)	144.5 (11.9)	87.4 (10.1)	101.1 (11.0)
	Median (Range)	130.5 (94-227)	143.5 (97-194)	84.5 (46-136)	93.5 (47-175)
1	Mean (S.E.)	101.1 (12.3)	111.6 (11.2)	66.3 (8.6)	72.1 (6.6)
	Median (Range)	88 (57-169)	101 (78-181)	63 (36-117)	65 (44-101)
	% Reduction Of Median MRP Compared With Start	**32.6 %**	**29.6 %**	**25.4 %**	**30.4 %**
2	Mean (S.E.)	96.5 (7.8)	97.5 (7.9)	66.9 (7.8)	75.8 (6.7)
	Median (Range)	96 (61-140)	90 (72-142)	55 (46-114)	76 (47-118)
	% Reduction Of Median MRP Compared With Start	**26.4 %**	**37.2 %**	**34.9 %**	**18.7 %**
3	Mean (S.E.)	85.9 (8.1)	91.0 (8.9)	59.3 (7.6)	67.8 (9.7)
	Median (Range)	91 (57-120)	92 (53-132)	53 (33-112)	68 (34-128)
	% Reduction Of Median MRP Compared With Start	**30.2 %**	**35.8 %**	**37.2 %**	**27.3 %**

Figure 5.2. Effect of 20mg oral indoramin on the median maximum resting anal canal pressure (MRP) in 10 healthy volunteers over a 3 hour period.

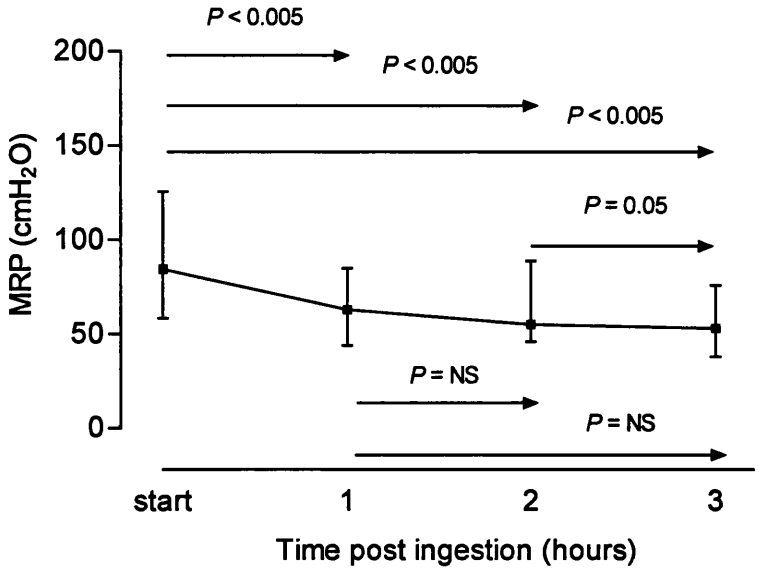


Figure 5.3. Effect of 4mg oral salbutamol on the median maximum resting anal canal pressure (MRP) in 10 healthy volunteers over a 3 hour period.

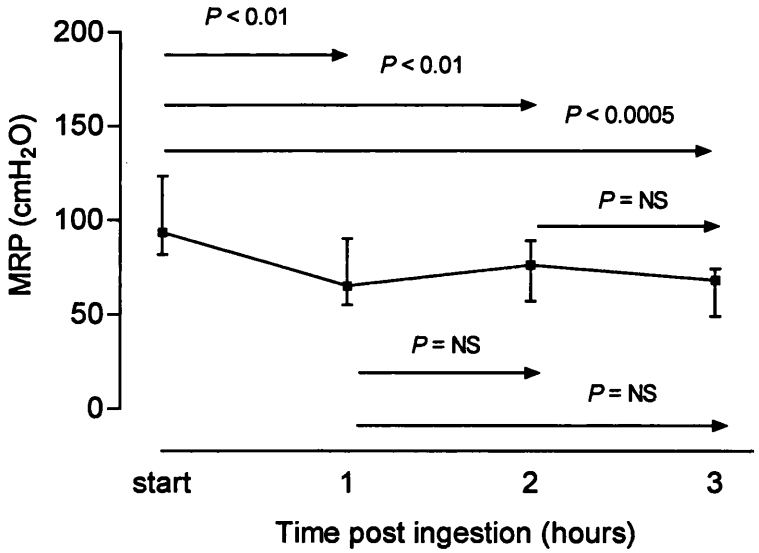


Figure 5.4. Effect of 20mg oral indoramin on the median maximum resting anal canal pressure (MRP) in 10 patients with chronic anal fissures over a 3 hour period.

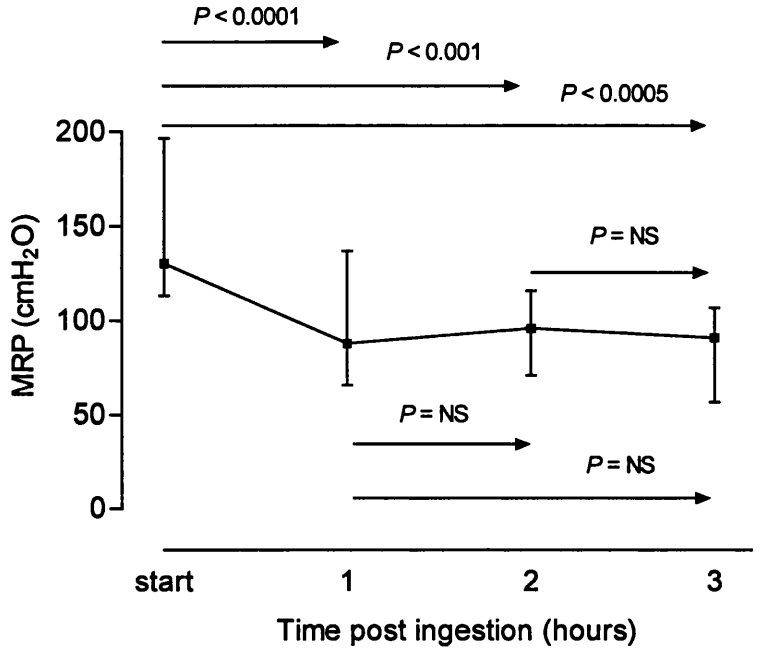


Figure 5.5. Effect of 4mg oral salbutamol on the median maximum resting anal canal pressure (MRP) in 10 patients with chronic anal fissures over a 3 hour period.

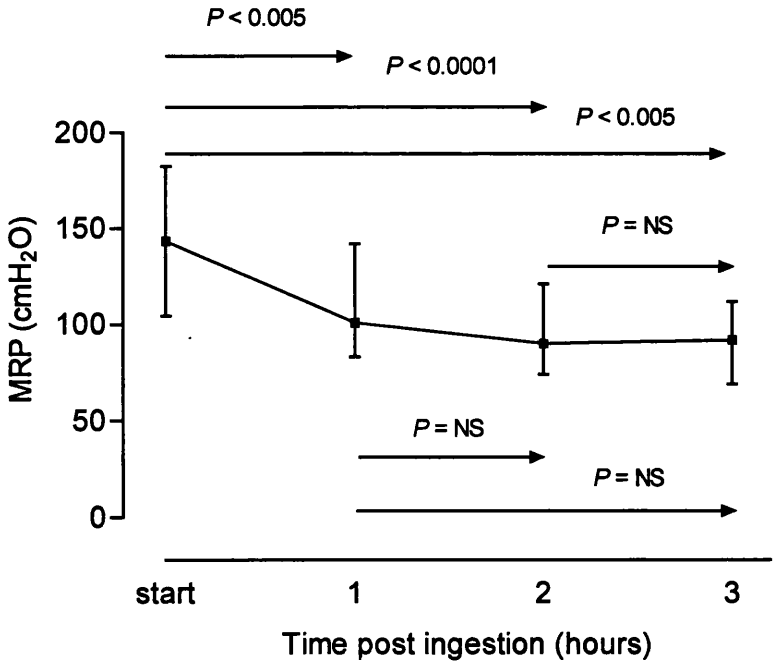


Figure 5.6. Effect of 20mg oral indoramin on the pulse rate (/minute) in 10 patients with chronic anal fissures and 10 control subjects measured over a 3 hour period.

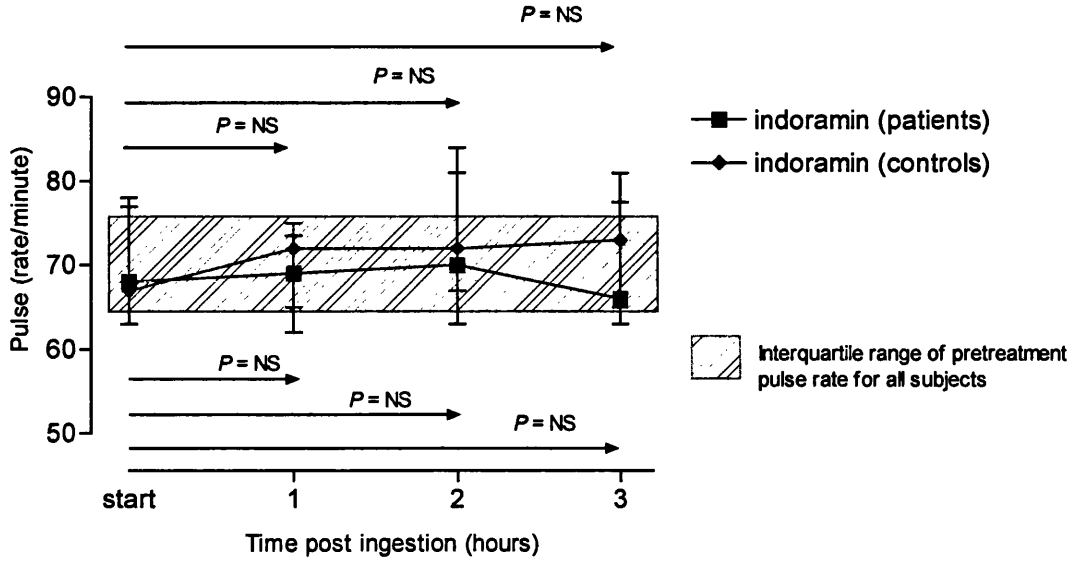


Figure 5.7. Effect of 4mg oral salbutamol on the pulse rate (/minute) in 10 patients with chronic anal fissures and 10 control subjects measured over a 3 hour period.

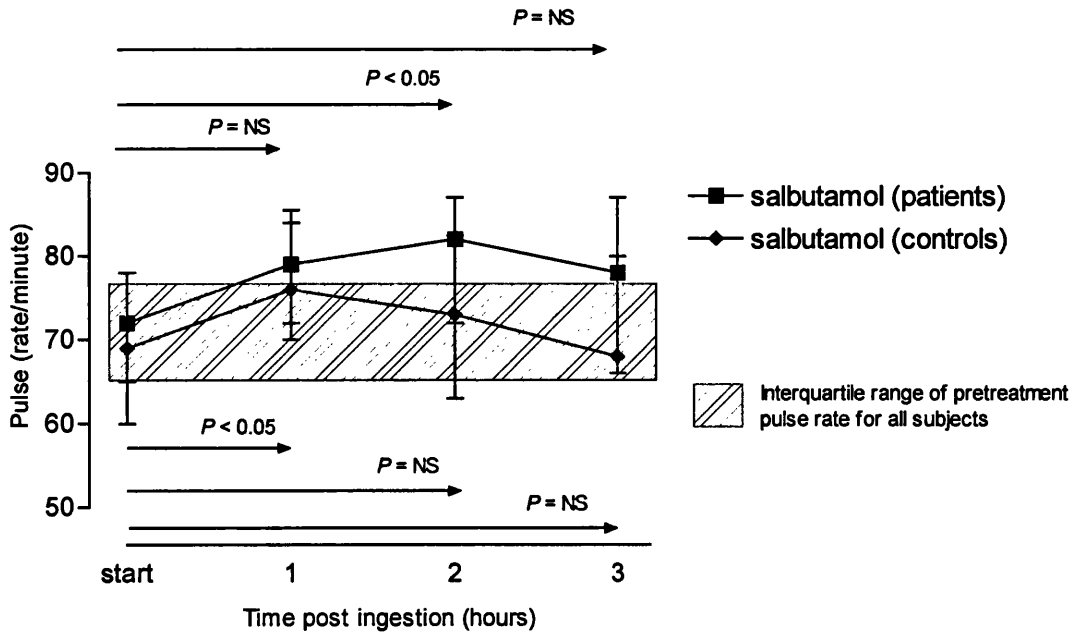


Figure 5.8. Effect of 20mg oral indoramin on the systolic blood pressure (mmHg) in 10 patients with chronic anal fissures and 10 control subjects measured over a 3 hour period.

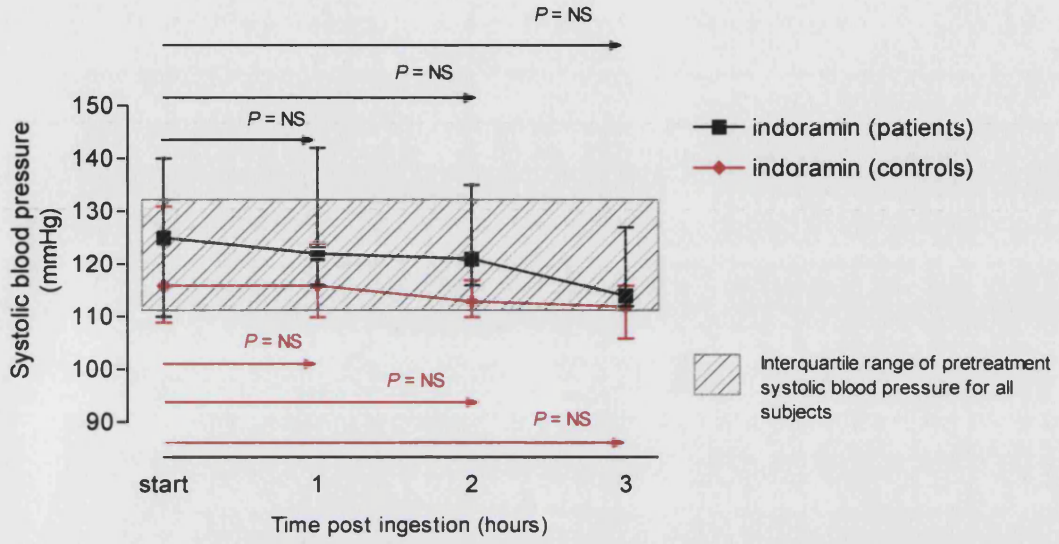
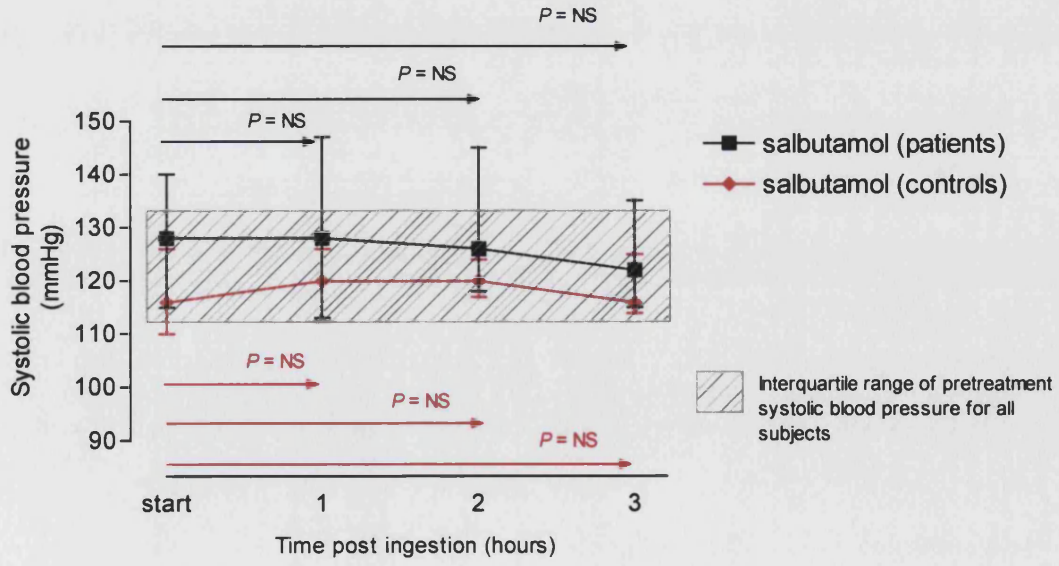


Figure 5.9. Effect of 4mg oral salbutamol on the systolic blood pressure (mmHg) in 10 patients with chronic anal fissures and 10 control subjects measured over a 3 hour period.



5.3. EFFECT OF TOPICAL INDORAMIN ON ANAL CANAL PRESSURE

Analysis of the results from the oral trial presented in this chapter was aimed at determining the safety of salbutamol and indoramin before investigating the therapeutic potential of using a topical formulation. Salbutamol was excluded from further analysis due to the high incidence of side effects demonstrated in 9 (45%) of 20 subjects. Since indoramin caused side effects in 3 (15%) of 20 subjects and there was no change in systolic blood pressure it was felt that a topical formulation should be investigated.

5.3.1. PATIENTS AND METHODS

Pilot Study

An initial pilot trial attempted to determine if a topical formulation of indoramin lowered the MRP. Indoramin is not freely soluble in water, but in paraffin. A topical preparation of indoramin was not commercially available; therefore pilot formulations of 30mg indoramin were mixed with paraffin/wool fat similar to the carrier used for GTN to a total volume of 1.2ml (*fig.5.10.*). These were applied to the anal verge in 2 subjects, a 36 year old female and 24 year old male, to determine whether this formulation like the oral preparation caused any tentative effect on the MRP by 1 hour. At 1cm from the anal verge the MRP reduced by 19% and 23%, and at 2cm from the anal verge the MRP was reduced by 34% and 40%. Since the highest anal canal pressures are in the lower anal canal and a topical formulation was being used the resting pressure measurements at 1 and 2cm from the anal verge was deemed

sufficient for analysis. These results prompted a randomised blinded trial was to determine the effect of topical indoramin in varied concentrations on the MRP. Measurements of MRP were made by a station manometry technique described in Appendix 1.

Figure 5.10. Indoramin (30 mg/ 1.2 ml) in a paraffin/ wool fat base (for topical application)

Randomised Trial

Eight healthy volunteers (median age 29.5, range 20-39 years, five males) were selected. Local ethical committee approval was obtained. Four topical preparations of indoramin (10mg, 20mg, 30mg, 40mg) were added and made up to a volume of 0.6 ml of paraffin/wool fat. A placebo preparation, of 0.6 ml of paraffin/wool fat, made a total of 5 doses each for 8 volunteers. This volume was selected at it was more manageable to apply to the anal verge. Each preparation was randomly allocated to a subject to make a total of five day-visits, with a minimum washout period of 18 hours, representing 3 half lives of indoramin. The participants and the investigators were blinded to the doses of the preparations. On each occasion the MRP was measured

prior to and then at 1 and 3 hours after the application of the agent. When a response was noted at either 1 or 3 hours the measurements were continued at 2-hourly intervals until the MRP returned to the pre-treatment level. If, by 7 hours post application there appeared to be a sustained reduction, the study was terminated. It was difficult to recruit more healthy volunteers. The differences within each dose were calculated with the *paired t test* after testing differences of MRP for normality. Any effect of formulated dose over placebo was determined with a one-tailed analysis of variance (*ANOVA*).

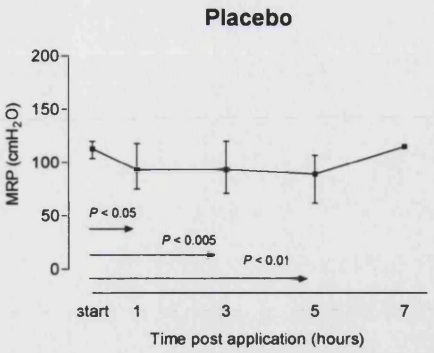
5.3.2. RESULTS

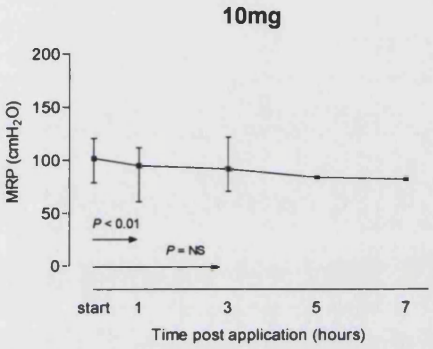
The data are shown in *Appendix 20* and *table 5.2.* with the mean (s.e.) and median (range) MRP shown, and the % reductions of median MRP. The results for each dose are also presented graphically as medians (interquartile ranges) (*fig.5.11*). At 1 hour placebo, 10mg and 20mg caused a significant reduction in the MRP. At 3 hours the placebo, 20mg and 30mg showed a significant reduction in MRP. At 5 hours placebo and 30mg achieved significant reduction in MRP. Because of the blinded nature of the study there was little effect on MRP at 5 hours of the 10mg, 20mg and 40mg doses and of all doses at 7 hours; as such there was insufficient data. Whilst these results appear to demonstrate effects from certain doses, comparison of the differences for each dose-effect at 1 hour and then at 3 hours showed no difference for any dose or an overall effect of any doses of topical indoramin over placebo (P= NS, *ANOVA*) (*figs. 5.12 & 5.13*).

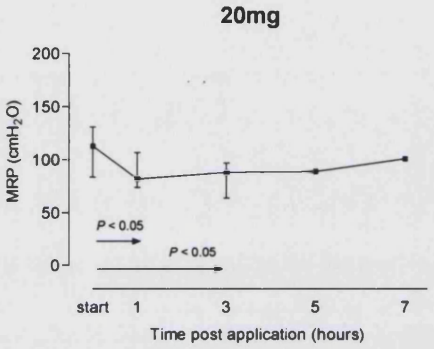
Table 5.2. The effect of placebo, 10mg, 20mg, 30mg and 40mg topical indoramin in a paraffin base on the MRP in 8 healthy volunteers.

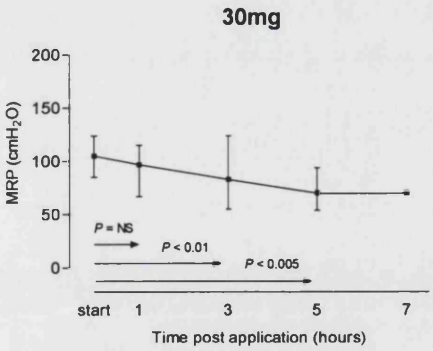
MRP (cmH_2O) at start (0), and 1, 3, 5 and 7 hours after application of topical agent		*PLACEBO*	*10 mg*	*20 mg*	*30 mg*	*40 mg*
0	Mean (s.e.)	110.0 (6.3)	104.8 (9.9)	114.4 (12.2)	105.6 (8.8)	105.5 (7.7)
	Median (range)	113 (74-135)	102 (78-159)	113 (79-182)	105 (73-147)	102 (77-147)
1	Number	8	8	8	7	8
	Mean (s.e.)	95.8 (8.1)	88.3 (9.9)	87.5 (8.1)	91.1 (11.8)	97.4 (9.9)
	Median (range)	93 (69-125)	95 (49-124)	82 (52-122)	97 (47-144)	90 (58-151)
	% Reduction Of Median MRP Compared With Start	**17.7 %**	**6.8 %**	**27.4 %**	**7.6 %**	**11.7 %**
3	Number	6	8	7	7	7
	Mean (s.e.)	88.8 (6.0)	96.5 (10.4)	79.7 (9.2)	91.0 (12.1)	102 (6.0)
	Median (range)	93 (68-109)	92 (62-143)	88 (35-109)	83 (51-127)	100 (83-132)
	% Reduction Of Median MRP Compared With Start	**17.7 %**	**9.8 %**	**22.1 %**	**20.9 %**	**1.9 %**
5	Number	6	2	5	6	2
	Mean (s.e.)	85.8 (8.4)	83.5 (11.5)	96.4 (6.4)	72.7 (8.7)	76 (8.0)
	Median (range)	89 (56-111)	84 (72-95)	89 (82-117)	70 (46-109)	76 (68-84)
	% Reduction Of Median MRP Compared With Start	**21.2 %**	**17.6 %**	**21.2 %**	**33.3 %**	**25.4 %**
7	Number	3	3	3	4	2
	Mean (s.e.)	108.3 (19.3)	85.0 (15.3)	90.3 (12.7)	70.3 (9.5)	88 (4.0)
	Median (range)	115 (72-138)	82 (54-123)	101 (65-105)	70 (48-94)	88 (84-92)
	% Reduction Of Median MRP Compared With Start	**-1.7%**	**19.6 %**	**10.6 %**	**33.3 %**	**13.7 %**

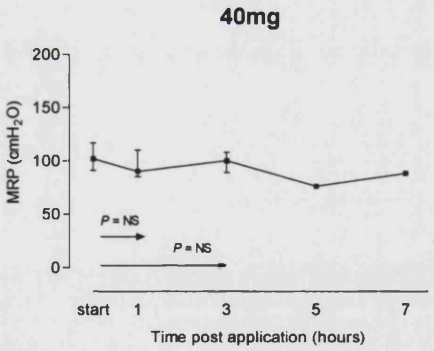
Figure 5.10. The effect of placebo, 10mg, 20mg, an 30mg and 40mg topical indoramin in 0.6ml paraffin base on the median MRP in 8 healthy volunteers. The error bars indicate the interquartile ranges and where shown assessment of significance can be determined. When no error bars are displayed the number of patients returning for assessment, judged by cessation of effect at the previous reading, was too few to determine a meaningful effect of the dose on MRP.











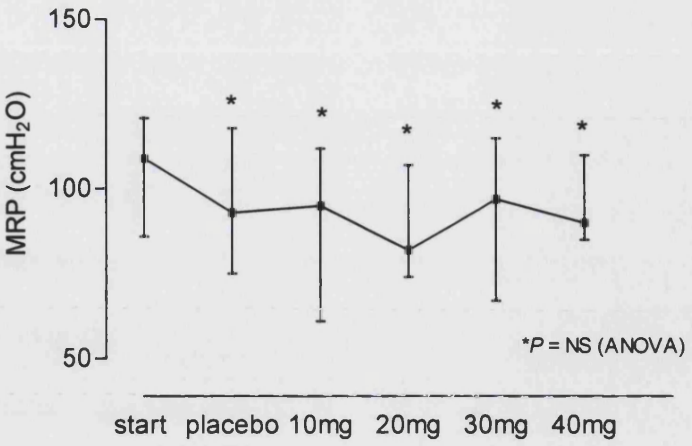


Figure 5.12. Effect of placebo and 10mg, 20mg, 30mg, and 40mg topical indoramin and the response of MRP of 8 volunteers at 1 hour post application [median (interquartile range)]. The "start value" indicates the MRP [median (interquartile range)] of the whole group pre-treatment. There was no effect of any dose of indoramin over placebo (*P* = NS, *ANOVA*).

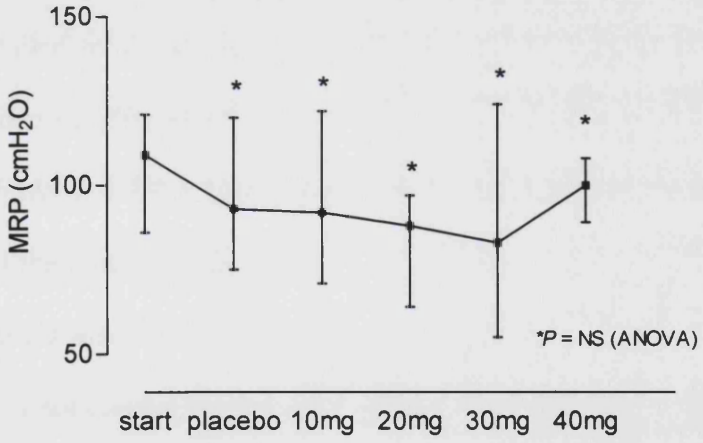


Figure 5.13. Effect of placebo and 10mg, 20mg, 30mg, and 40mg topical indoramin and the response of MRP of 8 volunteers at 3 hours post application [median (interquartile range)]. The "start" value indicates the MRP [median (interquartile range)] of the whole group pre-treatment. There was no effect of any dose of indoramin over placebo (*P* = NS, *ANOVA*).

5.4. DISCUSSION

The results with indoramin from the oral trial seem to validate the effects described by Pitt *et al.* (2000) who described a significant reduction in the MRP by 1 hour, which was sustained over the 3 hour study period. Whilst Ojo-Aromokudo *et al.* (1998) described a greater reduction with salbutamol in patients as compared to controls, this was not substantiated in this study. The magnitude of reductions in the MRP with salbutamol in patients compared with volunteers was no different at one hour. In this study the number of subjects was larger and dedicated anorectal manometric water perfused catheter assemblies were used as opposed to microballoons.

However while a reduction in MRP both patients and controls was observed Acheson *et al.* (2002) failed to show any effect of salbutamol over placebo on MRP in 15 healthy volunteers or offer an explanation for this observation. Regadas *et al.* (1993) observations have not been validated, they failed to explain why there is no upregulation of the β adrenoceptors in patients with haemorrhoids given that patients with haemorrhoids also have higher anal canal pressures that are also sustained. Furthermore it is not clear why there should be supersensitivity of the β adrenoceptor group alone as in animal hypertensive models it is the α adrenoceptors that are up regulated [Weishar *et al.* 1991].

While salbutamol reduced the MRP it was associated with side effects in nearly half of the subjects and in this respect indoramin was the preferred option for the

therapeutic trial. Whilst Pitt *et al.* (2000) did not describe any side effects in 13 subjects (including 6 volunteers) a subsequent oral trial in 14 patients with chronic anal fissures who were given 20mg indoramin showed side effects that included fatigue, dizziness, headache, dry mouth, nasal congestion and retrograde ejaculation [Pitt *et al.* 2001b].

Indoramin topical preparation did not differ from placebo in demonstrating an effective reduction in the MRP. This could be explained by the large sympathetic drive to the IAS not counteracted by increasing doses of indoramin. Indeed Frenckner and Euler (1975) demonstrated significant sympathetic contribution in maintaining the resting anal canal pressure. This was shown by comparing the anal canal pressure in patients after a high or low spinal anaesthetic, or bilateral pudendal blocks. Yamato and Rattan (1990) demonstrated that the α_1-agonist phenylephrine caused a dose-dependent rise in the resting IAS pressure, with a significantly higher response in the proximal part than in the distal part of the anal canal. This response was antagonised by prazocin, an α_1-antagonist. It is plausible that topical indoramin failed to affect the distal IAS tissue, where a reduction of IAS pressure is required to treat fissures.

The results presented so far indicate that whilst a 20mg dose of oral indoramin reduces MRP, it is not effective in the treatment of chronic anal fissures. Furthermore a topical preparation of indoramin, in a dose ranging from 10 to 40mg in a paraffin base, is not different from a placebo in reducing MRP. Oral salbutamol causes too many side effects to be considered as an effective agent for treatment of anal fissures.

CHAPTER SIX

VALIDATION OF AN ANIMAL MODEL OF HUMAN INTERNAL ANAL SPHINCTER

6.1. INTRODUCTION

Our understanding of the neurophysiology of the IAS is based on human and animal studies. *In vivo* work by Gaskell (1920) and Learmonth (1929) defined an excitatory sympathetic and inhibitory parasympathetic supply to the IAS. Stimulation of the hypogastric nerves in cats by Carlstedt *et al.* (1988), in dogs by Mizutani and Nakayama (1986) and in opposums by Shibamoto *et al.* (1994) led to adrenergically mediated contractile responses of the IAS. Yamato and Rattan (1990) studied the role of α-receptor subtypes in the opossum and concluded that α_2 adrenoceptors exert important neuromodulatory influences on rectoanal inhibitory reflex, while α_1 adrenoceptors may exert modulatory effects on the resting IAS tone. Early *in vitro* studies by Friedmann *et al.* (1968) and Parks *et al.* (1969) identified the α-adrenoceptor mediated contraction and β-adrenoceptor mediated relaxation of human IAS when investigating the action of noradrenaline, adrenaline, isoprenaline and acetylcholine. Later Burleigh *et al.* (1979) examined the relaxant response in human IAS muscle strips to electrical field stimulation (EFS). The identification of nitric oxide (NO) as an enteric inhibitory transmitter was made possible by Rattan and Chakder (1992) and Chakder *et al.* (1993), with work on opossum IAS muscle strips. Stebbing *et al.* (1998) used a guinea-pig model to demonstrate evidence of an inhibitory enteric pathway. Whilst studies on human IAS have evolved, the introduction of stapling techniques advanced restorative rectal excision which has impinged on the demand for human tissue. Interest in the pharmacological manipulation of smooth muscle tone of the IAS, for the treatment of anal fissures, has prompted search for suitable models for human IAS.

Rat Anococcygeus

The rat anococcygeus muscle was initially considered as Gibson and Gillespie (1973) demonstrated its contractility was sympathetically mediated. However Gillespie (1972) had previously shown that the rat does not have an anatomically definable IAS, and as Creed and Gillespie (1974) and Creed *et al.* (1975) showed that the rat anococcygeus does not have spontaneous myoelectrical activity and as such is an unsuitable model for investigation of the pharmacological properties of the IAS

Guinea-Pig IAS

Lim and Muir (1985) defined the mechanisms underlying the electrical and mechanical responses of the guinea-pig IAS to electrical field stimulation (EFS) and drugs. The IAS developed its own tone (3-4g), following initial stretch (1g) and spontaneous spike potentials were evident. In the absence of spike potentials, tone declined and disappeared. EFS (1-20Hz, 0.5ms, supramaximal voltage) produced inhibitory junction potentials and relaxed tone. These inhibitory junction potentials and relaxations were inhibited by apamin, tetrodotoxin but not atropine, phentolamine and hexamethonium. These results suggested work that could examine the relationship between the basal tone and the effects of EFS on relaxation of the IAS muscle strips could be undertaken.

Sheep IAS

Mundey *et al.* (2000) investigated the sheep IAS and demonstrated that muscle strips of sheep IAS develop intrinsic contractile tone following the application of stretch

tension. On EFS (1 - 20 Hz, 10 V pulse strength, 0.5 ms pulse width, 1 s every 180 s) transient relaxations were observed. The amplitude of the relaxations were frequency-dependent reaching a maximal response at 10-20 Hz and were inhibited by tetrodotoxin (0.3 μM). Neither atropine (0.3 μM) nor phentolamine (1 μM) affected control responses. The nitric oxide synthase inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME, 100 μM) and the selective inhibitor of soluble guanyl cyclase ODQ, (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one) (1 μM) completely inhibited the neurogenic relaxations and uncovered contractions that were abolished by 1 μM phentolamine and 0.1μM prazosin. The effect of L-NAME, but not that of ODQ, was partially reversed by the addition of L-arginine (1 mM), a substrate for nitric oxide synthesis. Sodium nitroprusside (10 nM - 10 μM) caused concentration-dependent inhibition of myogenic tone and this effect was significantly reduced by ODQ. Calcium-free Krebs-Henseleit solution also reduced myogenic tone by 85%. The authors concluded that EFS of sheep IAS muscle strips caused a transient relaxation of myogenic tone that appeared to involve NO from non-adrenergic, non-cholinergic nerves and, to a lesser degree, noradrenaline from sympathetic nerves. Griffin *et al.* (2001), using the same model, demonstrated that L-arginine did not affect either the resting tone or magnitude of electrically induced contractions; furthermore they showed that L-arginine prevented L-NAME-induced inhibition of electrically evoked relaxations. These studies provided adequate validation that sheep IAS muscle strips could be substituted for human IAS muscle strips in pharmacological investigation. Jonas *et al.* (2001c) used this model to show that whilst glyceryl trinitrate and diltiazem each caused a reduction of sheep IAS tone by 70%, a combination of these drugs caused abolition of IAS tone irrespective of the order in which they were added.

Porcine IAS

O'Kelly *et al.* (1992) investigated the possibility of the porcine IAS as a suitable animal model of the human IAS. Porcine IAS muscle strips from Landrace pigs were mounted for isometric tension, with an initial load of 1g applied to provide tone. Electrical stimulation (10V, 0.5ms duration and 8 pulses per second) of the NANC (non adrenergic, non cholinergic) inhibitory nerves produced terodotoxin sensitive relaxations. Sodium nitroprusside also relaxed the muscle strips in a concentration-dependent manner; after which the tone could be re-established with histamine. The inhibitory NANC responses were partially inhibited by L-N-monomethylarginine (L-NMMA) ($5x10^{-5}$ M) and abolished by oxyhaemoglobin as well as L-nitroarginine (L-NOARG) (10^{-6}-10^{-4} M). The effects of L-NMMA and L-NOARG were reversed by equimolar concentrations of L-arginine but not D-arginine. Cook *et al.* (1999b) determined, in a similar experimental manner, that tone and spontaneous activity were dependent upon extra-cellular calcium and flux across the cell membrane, through L-type calcium membrane channels, whilst agonist-induced contractions were dependent mainly on release of calcium from the intracellular sarcoplasmic reticulum. Furthermore the addition of nifedipine, an L-type calcium channel antagonist which blocks entry of calcium into smooth muscle cells, abolished tone and spontaneous activity.

It has been established that nitric oxide is the predominant neurotransmitter that induces non-adrenergic non-cholinergic induced relaxation of the internal anal sphincter (IAS). What is not clear is how the effect of other neurotransmitters or intracellular messengers that may be less important in this response modulate this

relaxation. To explore these interactions it was therefore proposed to investigate the actions of purinergic and alpha adrenoceptor antagonists on the relaxant induced responses to electrical field stimulation. ATP is commonly a co-transmitter with vasoactive intestinal polypeptide and nitric oxide in enteric inhibitory neurones [Hoyle, 1996a]. To date the effect of an inhibition of the IP_3 induced cellular contraction through α_1-adrenoceptor antagonism and its modulation had not been investigated.

Botulinum toxin is an endopeptidase which blocks acetylcholine release at the neuromuscular junction of alpha motor neurones, at gamma neurones in muscle spindles and in all parasympathetic and cholinergic postganglionic sympathetic neurones. Jones *et al.* (2002a) recently described the mechanism of action of botulinum toxin on the IAS. The electrically field stimulated responses (EFS) to isolated porcine IAS tissue were compared before and after incubation with botulinum toxin. It was found that treatment with this agent increased myogenic tone by 38%. Whilst the EFS-induced relaxations were unaffected, the EFS-induced contractions were significantly reduced. It was concluded that the blockade of sympathetic output by botulinum toxin probably outweighed its effect on increased myogenic tone and that botulinum toxin blocked sympathetic nerves distal to their ganglia, by reducing noradrenaline release at the neuromuscular junction, but had no effect on nitregic transmission.

The aims of studies presented here were to determine if the physiological parameters of guinea-pig and porcine IAS muscle strips are comparable to human IAS in order to

affirm if these tissues could be substituted satisfactorily for human IAS in further studies.

6.2. METHODS

Male guinea pigs (Dunkin Hartley, Charles River; weight 275-375g) were killed by cervical dislocation. The abdomen was opened, internal viscera excised, and the pelvic floor structures were removed and transported in modified Kreb's solution to the laboratory where the IAS tissue was dissected free. The sphincter complexes of female Landrace pigs (live weights 80-120kg) killed at the abbatoir in accordance with approved methods (captive bolt stunning, and exsanguination) were transported en bloc in modified Kreb's solution to the laboratory where the IAS tissue was dissected free.

Muscle strips of IAS (guinea-pig: 4mm x 1mm, porcine 5-10mm x 2mm) were suspended in organ-baths containing modified Krebs' solution. One end of the preparation was attached to a rigid support and the other, using silk thread, to an isometric force-displacement transducer (Biopac TSD 125 C). The data were presented with "Acquire software (Acqknowledge 2.0)" computer software, through a MPW100 data acquisition system (Biopac). Preparations were preloaded with 0.5-2.0g and were allowed to equilibrate for at least 50 min before any drugs were added. Where tissues were electrically stimulated (Experimetria S02 stimulator) the following stimulation parameters were used: pulse width (0.3 ms, biphasic);

frequency (0.5 - 32 Hz); train length (10 s); interval between trains (110 s) and voltage (supramaximal, 60 V).

The concentration–response curves in each specimen were constructed by addition of the following drugs: histamine, phenylephrine, isoprenaline, GTN, acetylcholine, L-NAME, ATP, and suramin. The organ-bath concentrations are shown in the results. Relaxant or contractile responses were allowed to plateau before the concentrations were increased. The contractile responses in the guinea pig preparations were measured as percentage increase above basal tone, and the contractile responses in the porcine tissues were measured as percentages of total maximal contraction. The relaxant responses were measured as percentages of basal levels. The results for the effects of L-NAME on the EFS-induced relaxation of tissue are represented in absolute relaxant amplitudes. The effects of the purinoceptor antagonist, suramin on the relaxant response to EFS were investigated and the results and were expressed as percentages of basal levels. Regadas *et al.* (1993) investigated the actions of adrenoceptor antagonists on the human IAS, and it was appropriate to study the responses to similar agents in the porcine model. The effects of prazosin and indoramin on histamine and phenylephrine response curves were investigated. The results were expressed as percentages of total maximal contraction. The role of botulinum toxin exerted on the acetylcholine-induced relaxation of porcine IAS was investigated, as in botulinum toxin inhibits the release of acetylcholine at the motor nerve ending striated muscle. Similarities of action in the smooth muscle of the IAS were sought. These results were expressed as the extent of relaxation from basal levels. Statistical analysis was conducted using the *paired t test* for paired observations.

6.3. RESULTS

GUINEA PIG IAS

The results are shown in *Appendices 21 to 23* and *figures 6.1. to 6.3.* When placed into the organ bath, the IAS had no tone as measured with the force transducer. The preparation was then subjected to a tension of 1g by stretching the tissue. The tone was maintained at this level throughout the experiments. In 6 preparations the tone was significantly raised by histamine (1x 10^{-5}, 3 x 10^{-5}, 1x 10^{-4}M) (*fig.6.1.*). There was no significant difference in the basal tone in 6 different muscle strips after the application of atropine (1x 10^{-6}M; 0.25 ± 0.08 g *versus* 0.30 ± 0.09 g, mean ± s.e.). Furthermore there was no significant effect of the adrenoceptor agonist methoxamine (1x 10^{-4}M) when applied to 4 muscle strips (0.23 ± 0.04 g *versus* 0.39 ± 0.11g).

The response to EFS (8Hz) was initial relaxation followed by rebound excitation (*fig.6.2.*). There was a linear relationship between tension (increased by histamine) and relaxant response (indicated here by the area of relaxation) at levels of tone up to 1 g ($y=8.08x-3.64$, $r^2=0.98$, d.f.=2) (*fig.6.3.*).

PORCINE IAS

The results are shown in *Appendices 24 to 36* and *figures 6.4. to 6.17.* Histamine (1x10^{-7}M -1x10^{-3}M) induced a concentration-dependant contraction (*fig.6.4.*); the mean maximal contraction was 2.4 ± 0.5g (mean, s.e.mean) above basal tones. Phenylephrine (an α_1-adrenoceptor agonist) induced a concentration-dependent contraction of the tissue in (1x10^{-8} – 1x10^{-4} M) (*fig.6.5.*). The mean maximal

contraction was 4.0 ± 0.6g (mean, s.e.mean). Agonism of the β-receptors with isoprenaline ($1x10^{-7}$ – $1x10^{-3}$ M) resulted in relaxation (*fig.6.6.*). The addition of GTN also caused relaxation of the IAS in a dose-dependent manner ($2.2x10^{-4}$, $6.6x10^{-4}$, $2.2x10^{-3}$ M) (*fig.6.7.*). Acetylcholine ($1x10^{-3}$M) induced a rapid decrease in basal tone that was transient (*fig.6.8A.*) and in the presence of L-NAME ($1x10^{-4}$M) this response was significantly decreased ($P<0.05$, *paired t test*) (*fig.6.8B.*). ATP ($1x10^{-3}$M) induced a sustained decrease in basal tone (*fig.6.9A.*). In the presence of suramin ($3x10^{-4}$M), this response was significantly increased ($P<0.05$, *paired t test*) (*fig.6.9B.*).

EFS produced an initial rapid relaxation followed by a rebound excitation. This response was demonstrated across a broad range of frequencies (0.5–32Hz) (*fig.6.10.*), with a notable increase in the relaxant response as the frequency of stimulation was increased (*fig.6.11.*). L-NAME ($1x10^{-4}$M) abolished the EFS-induced relaxation, that was less notable at lower stimulation frequencies (*fig. 6.12.*). Suramin ($3x10^{-4}$ M) did not significantly effect the relaxant response to EFS (0.5-32 Hz) (*P = NS, paired t test*) (*fig. 6.13.*). The effect of prazosin ($1x10^{-7}$ M) on the relaxant response to EFS (0.5-32 Hz) demonstrated a significant increase as compared with the control preparation for the frequencies above 0.5 Hz (*$P < 0.05$, paired t test*) (*fig. 6.14.*).

For the experiment investigating the effect of prazosin ($1x10^{-6}$ M) on the histamine induced contraction an initial load of 2 g was applied, and after equilibration (45 min), by which time the tone had dropped to 0.8 ± 0.3 g (n = 3, mean ± s.e. mean), histamine was added cumulatively to provide a bath-concentration from $1x10^{-7}$ M - $1x10^{-4}$ M. Prazosin ($1x10^{-6}$ M) resulted in a shift of the histamine dose response curve

(fig. 6.15.) to the right. This effect was more demonstrable in concentrations of histamine above $1x10^{-6}$ M.

For the experiment investigating the effect of indoramin ($1x10^{-7}$ M) on the phenylephrine induced contraction initial load of 2 g was applied, and after equilibration (45 min), by which time the tone had dropped to 1.1 ± 0.2 g (n = 7, mean ± s.e. mean), phenylephrine was added cumulatively to provide a bath-concentration from $1x10^{-8}$ M - $1x10^{-4}$ M. Indoramin ($1x10^{-7}$ M) reduces the phenylephrine induced contraction of the IAS across the concentrations ($1x10^{-8}$ M - $1x10^{-4}$ M). For this antagonism the effect is indicated by the following relationship: pK_B = 8.3 ± 0.33. *(fig.6.16.)*

Incubation of IAS muscle strips with one unit of botulinum toxin for one hour did not significantly alter the relaxant response induced by acetylcholine ($1x10^{-3}$ M) *(P = NS, paired t test) (fig.6.17.).* The relaxant response prior to incubation with botulinum toxin was 43.9 ± 8.1 % (n=3, mean, s.e.mean.), and after incubation was 44.3 ± 9.2 % (n=3, mean, s.e.mean.).

6.4. DISCUSSION

The aim of the work presented in this chapter was to investigate the suitability of an animal model with physiological similarities to human IAS tissue.

Guinea pig IAS muscle strips maintain an applied tone, as demonstrated after preloading with 1g. It was not possible to confirm in the *in vitro* environment Frenckner and Ihre's (1976) observations of the existence of a tonic sympathetic discharge to the guinea pig IAS as isolated IAS muscle strips are devoid of a sympathetic input. Lim *et al.* (1985) demonstrated that adrenergic nerves in this species play little part in maintaining sphincteric tone and showed that it was maintained by fast action potentials, unlike that of human tissue. Histamine caused a dose dependent increase in basal tone which is in contrast to Burleigh *et al.* (1979) and Burleigh and D'Mello's (1983) observations in human IAS tissue where histamine caused a relaxant response. Rae and Muir (1996) investigated the neuronal mediators of inhibitory junction potentials and relaxation in the guinea-pig IAS and showed that EFS produced similar relaxations, and that nitric oxide was probably a transmitter that was responsible in part for this, the other being ATP or a closely related analogue.

In the porcine IAS a spontaneous tone could be maintained for a considerable time after loading. Histamine, through H_1 receptors, and phenylephrine, through α_1-adrenoceptors, induced a concentration dependent contractile response in porcine IAS muscle strips. Whether these receptors are located on the smooth muscle membrane can be determined by studying the effect of α_1, $H_1$and H_2 antagonists, such as indoramin, mepyramine, and ranitidine respectively. Alpha-adrenergic mediated cellular contraction results in the release of calcium from the intracellular sarcoplasmic reticulum, mediated via IP_3 [Cook *et al.,* 1999b]. From these data it is not possible to determine which of the two receptors are more potent at inducing contraction. In the human IAS, histamine induced a relaxant response, and was

believed to be responsible for NANC mediated relaxation of the IAS [Burleigh *et al.*, 1979], but has since been discounted.

The non-specific β-adrenoceptor agonist, isoprenaline, induced a relaxant response, which is similar to observations by Parks *et al.* (1969). This is mediated through cAMP with the resultant return of calcium to the sarcoplasmic reticulum. The effect of GTN on isolated muscle strips were investigated by in sheep IAS muscle strips by Jonas *et al.* (2001c) who identified dose dependent relaxations after addition of GTN. In the porcine model the dose dependent relaxations were similar as doses $2.2x10^{-4}$, $6.6x10^{-4}$ and $2.2x10^{-3}$ M caused a mean relaxation of 14.9%, 28.7% and 35.3% respectively. Burleigh *et al.* (1979) demonstrated that acetylcholine in human tissue induced a relaxant response, and in porcine a rapid transient decrease in tone was noted with $1x10^{-3}$ M. The abolition of the acetylcholine mediated relaxation by L-NAME suggests that the muscarinic relaxant pathways involve NO as a transmitter. The effect of ATP on the human IAS is negligible. However, in the porcine IAS it induced a sustained relaxation, which was enhanced by the purinoceptor antagonist suramin. The reason for this is unclear, but suramin is an inhibitor of ecto-ATPase and is also an antagonist of some P2 receptors. However, while suramin potentiated the responses to applied ATP in this study, it had no signifcant effect on responses to EFS, implying that under these conditions ATP does not contribute significantly to the inhibitory neuromuscular transmission

Nitric oxide plays a significant role in the relaxant response of the human IAS and this was similar in porcine tissue, demonstrated by the action of GTN, and the inhibitory effect of L-NAME on the relaxant amplitude induced by EFS. Although

nitric oxide is the predominant neurotransmitter in the porcine IAS, it is not solely responsible for inducing relaxation, as there was still some relaxation on EFS after the addition of L-NAME.

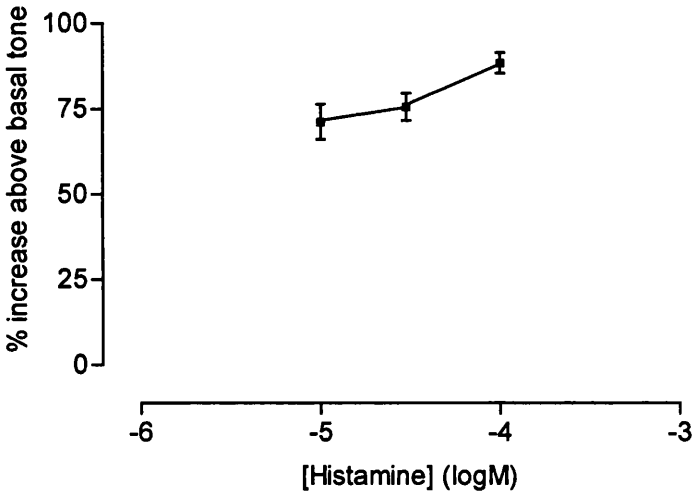
The rightward shift of indoramin on the concentration-response curve of phenylephrine suggests that indoramin acts through α_1 adrenoceptors as indoramin reduces the phenylephrine induced contraction of the IAS. Prazosin also reduces the histamine induced contraction of the IAS. Furthermore the increase in the relaxant response to EFS of frequencies greater than 0.5 Hz up to 32 Hz indicate that addition of α_1 adrenoceptor antagonists with nitric oxide donors may have a synergistic effect reducing IAS tone in the human IAS muscle strips.

The failure of botulinum toxin to reduce acetylcholine induced relaxation of porcine IAS muscle strips may be due to several reasons. Firstly the incubation period may not be long enough. Mackenzie *et al.* (1982) demonstrated a reduction in the twitch response of guinea-pig ileum, which is mediated by acetylcholine, after only two hours of incubation with botulinum toxin. However in humans the reduction of anal canal pressure after injection of botulinum toxin into the IAS is not demonstrable until after a few days. Secondly the dose may not be sufficient to counteract acetylcholine induced relaxation of the IAS. Work by Jones *et al.* (2002a) has since eluded to the effect of botulinum toxin on the porcine IAS, through blockade of sympathetic nerves distal to their ganglia.

This work shows that with the exception of histamine, causing contraction and ATP inducing relaxation, the neural properties of the porcine IAS showed similar features

to human tissue, as presented in the introduction of this thesis. Furthermore the porcine anal sphincter complex is structurally similar to the human, and pigs are phylogenetically more closely related to humans than rodents (rats) or hystricomorphs (guinea pigs). Hence the porcine IAS was regarded a suitable model of human IAS to pursue the aims of this work.

Figure 6.1. Effect of histamine on the basal tone of guinea-pig IAS isolated muscle strips. Points represent the mean and the vertical lines s.e.mean.



[Histamine] M	Number of muscle strips	% Increase in basal tone	
		mean	s.e.mean
$1x10^{-4}$	4	88.5	3.0
$3x10^{-5}$	6	75.7	4.0
$1x10^{-5}$	4	71.3	5.1

Figure 6.2. Diagramatic representation of the initial relaxant effect of electrical field stimulation (EFS 0.3 ms, 8 Hz, 60 V, train length 10 s, interval 110 s) on the tone of isolated guinea pig IAS muscle strip.

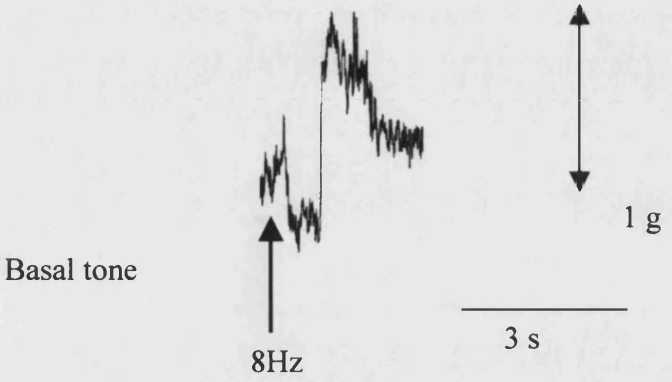


Figure 6.3. Representation of the dependency of the relaxant response on background tone in guinea pig internal anal sphincter (IAS) after electrical field stimulation (EFS: 0.3 ms, 8 Hz, 60 V, train length 10 s, interval 110 s). There is a linear relationship between tension (increased by histamine) and relaxant response (indicated here by the area of relaxation) at lower levels of tone for the tissue.

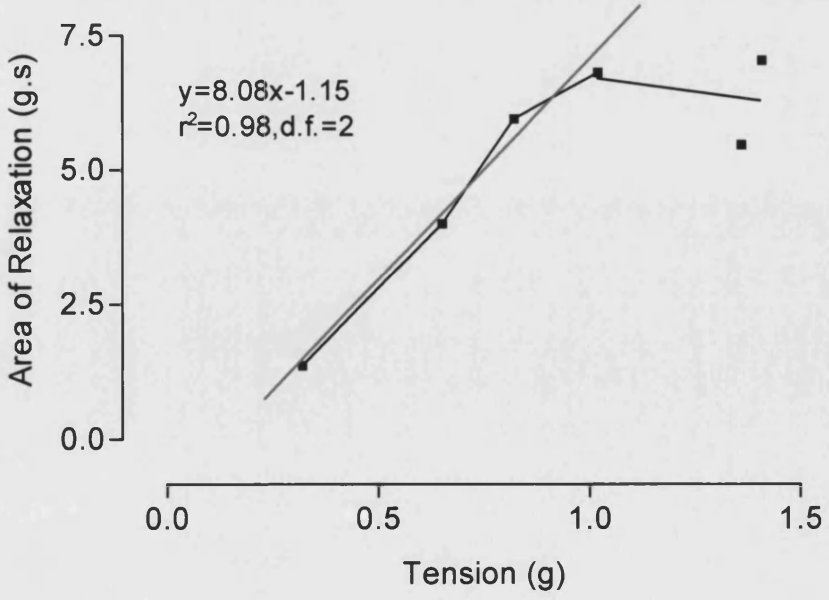
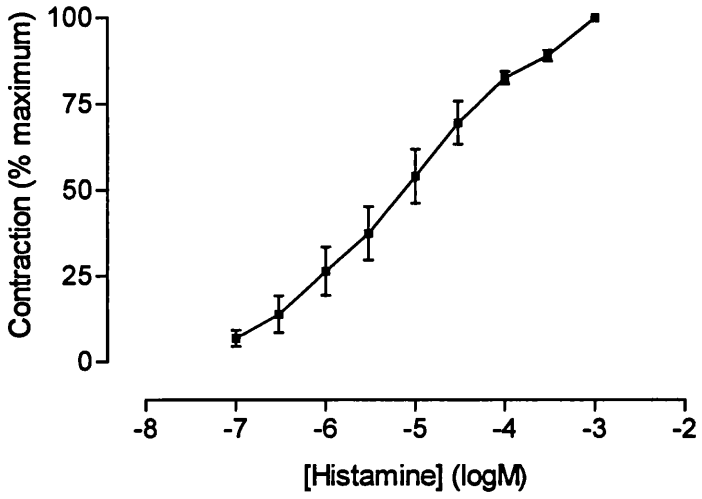
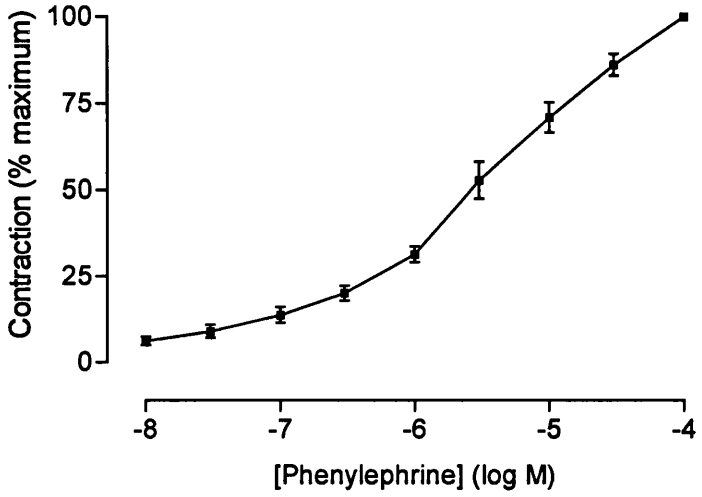


Figure 6.4. Histamine concentration-response curve in porcine internal anal sphincter (IAS). Points represent the mean and vertical lines s.e.mean.



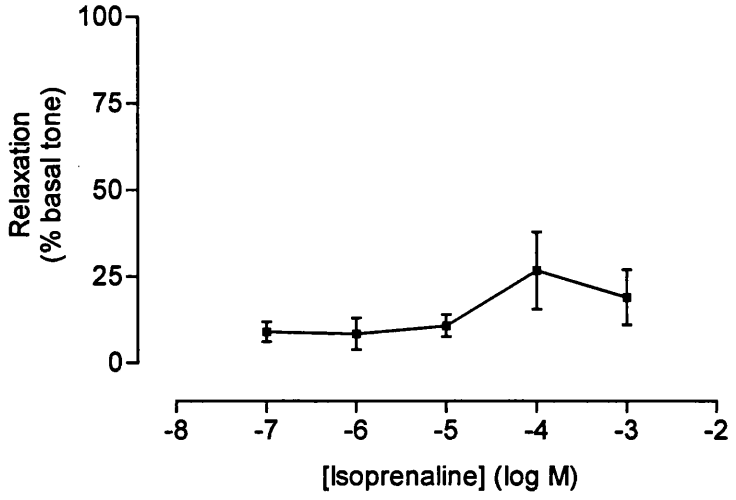
[Histamine] M	Number of muscle strips	Contraction (% maximum)	
		mean	s.e.mean
$1x10^{-7}$	3	6.9	2.4
$3x10^{-7}$	3	13.8	5.4
$1x10^{-6}$	3	26.4	7.1
$3x10^{-6}$	3	37.4	7.8
$1x10^{-5}$	3	54.1	7.8
$3x10^{-5}$	3	69.6	6.3
$1x10^{-4}$	3	82.7	1.8
$3x10^{-4}$	3	89.1	1.5
$1x10^{-3}$	3	100.0	0.0

Figure 6.5. Phenylephrine concentration-response curve in porcine internal anal sphincter (IAS). Points represent the mean and vertical lines s.e.mean.



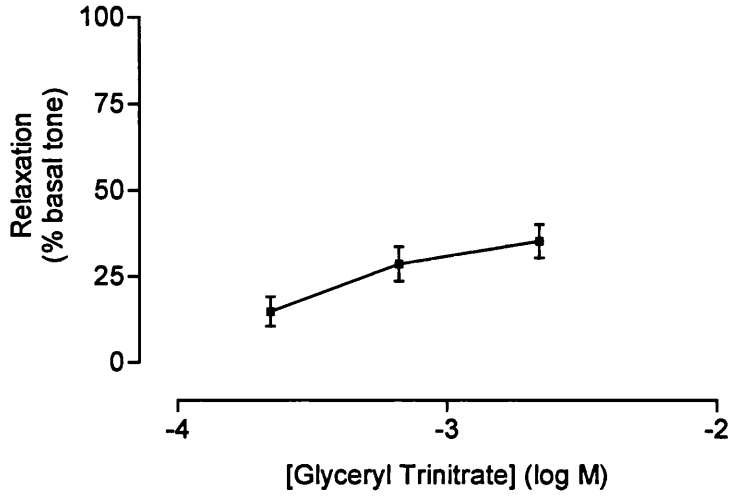
[Phenylephrine] M	Number of muscle strips	Contraction (% maximum)	
		mean	s.e.mean
$1x10^{-8}$	7	6.3	1.2
$3x10^{-8}$	7	9.1	1.9
$1x10^{-7}$	7	13.8	2.2
$3x10^{-7}$	7	20.1	2.2
$1x10^{-6}$	7	31.4	2.3
$3x10^{-6}$	7	52.9	5.3
$1x10^{-5}$	7	71.0	4.4
$3x10^{-5}$	7	86.2	3.1
$1x10^{-4}$	7	100.0	0.0

Figure 6.6. Isoprenaline concentration-response curve in porcine internal anal sphincter (IAS). Points represent the mean and vertical lines s.e.mean.



[Isoprenaline] M	Number of muscle strips	Relaxation (% basal tone)	
		mean	s.e.mean
$1x10^{-7}$	3	9.0	2.9
$1x10^{-6}$	3	8.5	4.6
$1x10^{-5}$	4	10.8	3.2
$1x10^{-4}$	5	26.8	11.1
$1x10^{-3}$	5	19.0	7.9

Figure 6.7. Relaxant effect of glyceryl trinitrate (GTN) ($2.2x10^{-4}$-$2.2x10^{-3}$ M) on the basal tone of porcine internal anal sphincter (IAS) at differing basal tones (0.72-8.73g) as raised by phenylephrine ($1x10^{-6}$-$1x10^{-4}$ M). Points represent the mean and vertical lines s.e.mean.



[GTN] M	Number of muscle strips	Relaxation (% basal tone)	
		mean	s.e.mean
$2.2x10^{-4}$	23	14.9	4.2
$6.6x10^{-4}$	22	28.7	5.0
$2.2x10^{-3}$	23	35.3	4.8

Figure 6.8A. Effect of acetylcholine ($1x10^{-3}M$) on the basal tone of porcine internal anal sphincter (IAS). Note the transient relaxant effect of the drug on the tissue.

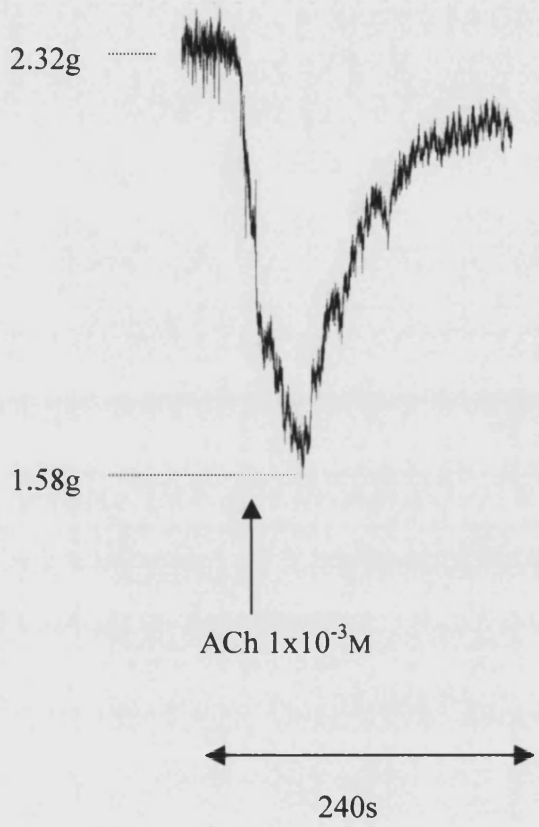
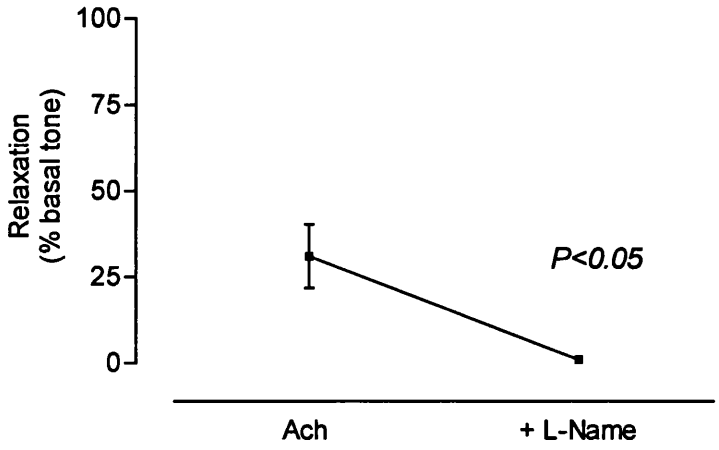


Figure 6.8B. The effect of L-NAME ($1X10^{-4}M$) on the acetylcholine ($1X10^{-4}M$) induced relaxation of porcine internal anal sphincter (IAS) (n=7). Points represent the mean and vertical lines s.e.mean. L-NAME ($1X10^{-4}M$) caused a significant reduction in the relaxant response of acetylcholine ($1X10^{-4}M$) induced relaxation ($P<0.05$, *paired t test*).



	Number of muscle strips	Relaxation (% basal tone)	
		mean	s.e.mean
Ach	7	31.1	9.2
Ach + L-Name	7	1.0	0.3

Figure 6.9A. Effect of ATP ($1x10^{-3}M$) on the basal tone of porcine internal anal sphincter (IAS). Note the sustained decrease in basal tone in the tissue.

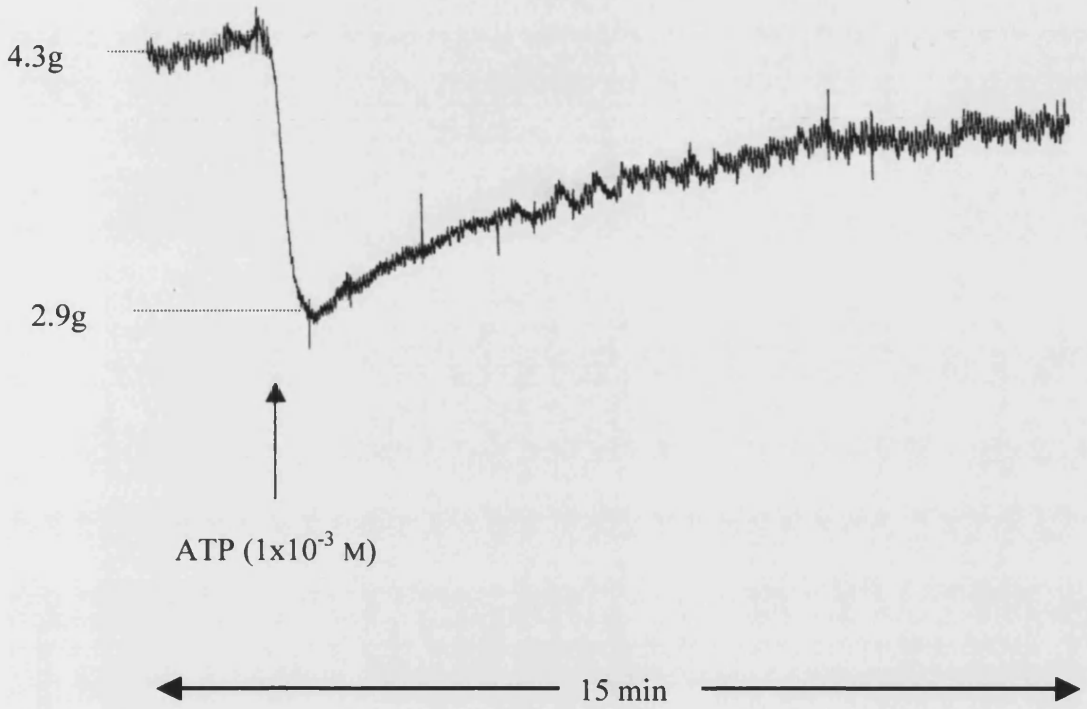
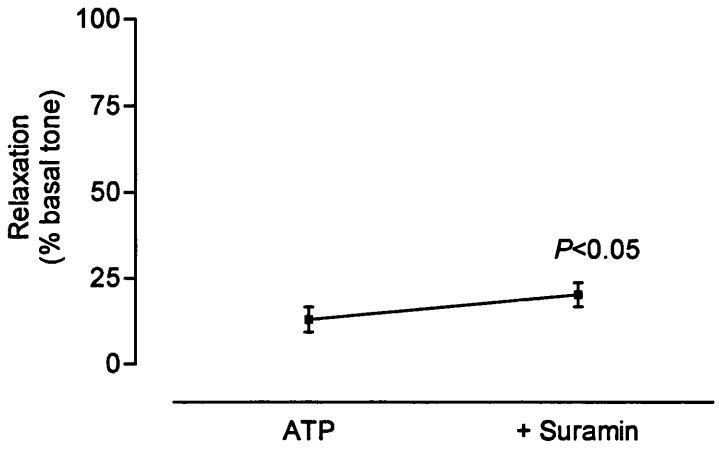


Figure 6.9B. The effect of suramin ($3X10^{-4}M$) on the ATP ($1X10^{-3}M$) induced relaxation of porcine internal anal sphincter (IAS) (n=7). Points represent the mean and vertical lines s.e.mean. Suramin ($3X10^{-4}M$) caused a significant increase in the relaxant response of ATP ($1X10^{-3}M$) ($P<0.05$, *paired t test*).



	Number of muscle strips	Relaxation (% basal tone)	
		mean	s.e.mean
ATP	7	13.1	3.7
ATP + suramin	7	20.3	3.5

Figure 6.10. Effect of frequency of stimulation on relaxant and contractile response of porcine internal anal sphincter (IAS). (EFS: 0.3 ms, 0.5-32 Hz, 60 V, train length 10 s, interval 110 s).

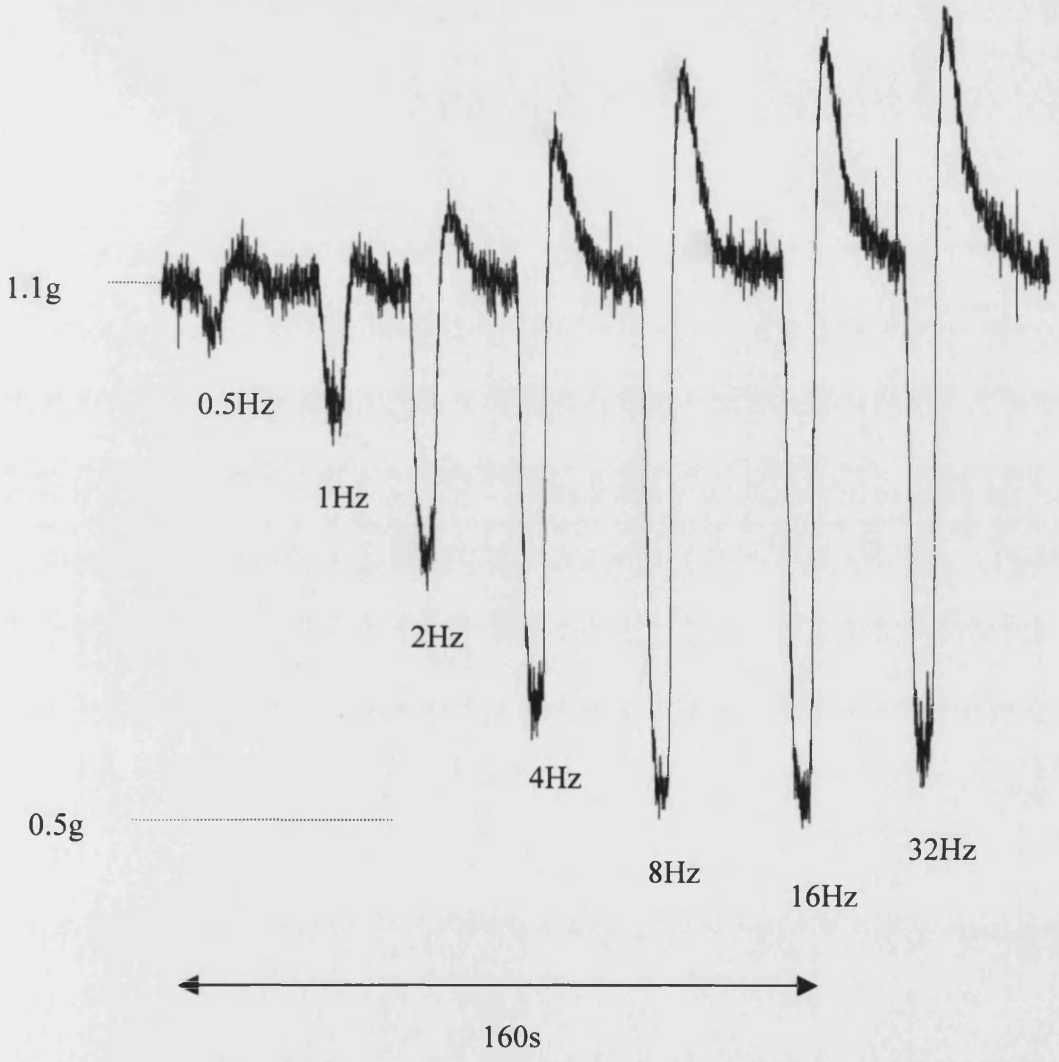


Figure 6.11. Effect of electrical field stimulation (EFS: 0.3 ms, 0.5-32 Hz, 60 V, train length 10 s, interval 110 s) on the relaxant amplitude of the porcine internal anal sphincter. The points represent mean values and the vertical bars s.e.mean. (n=9). Note that the abscissae are on a logarithmic scale.

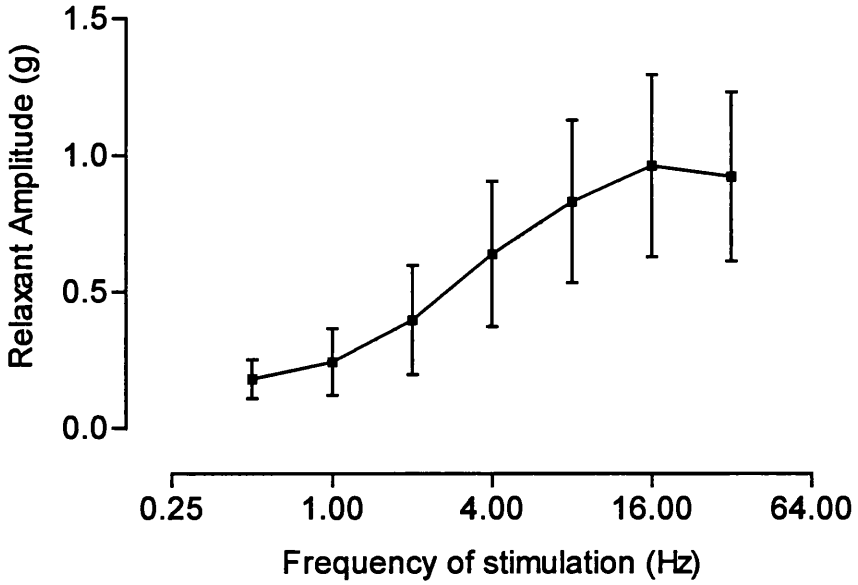
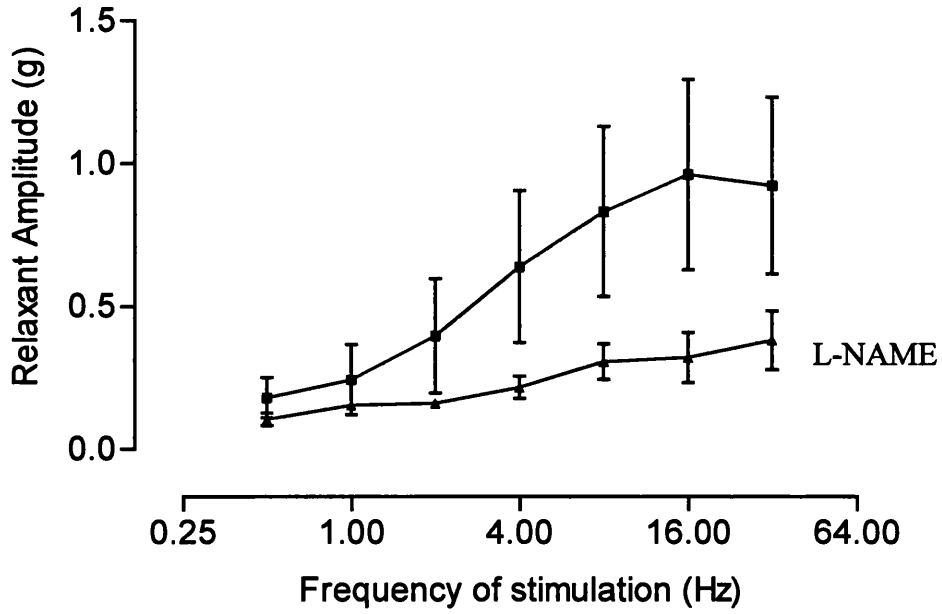
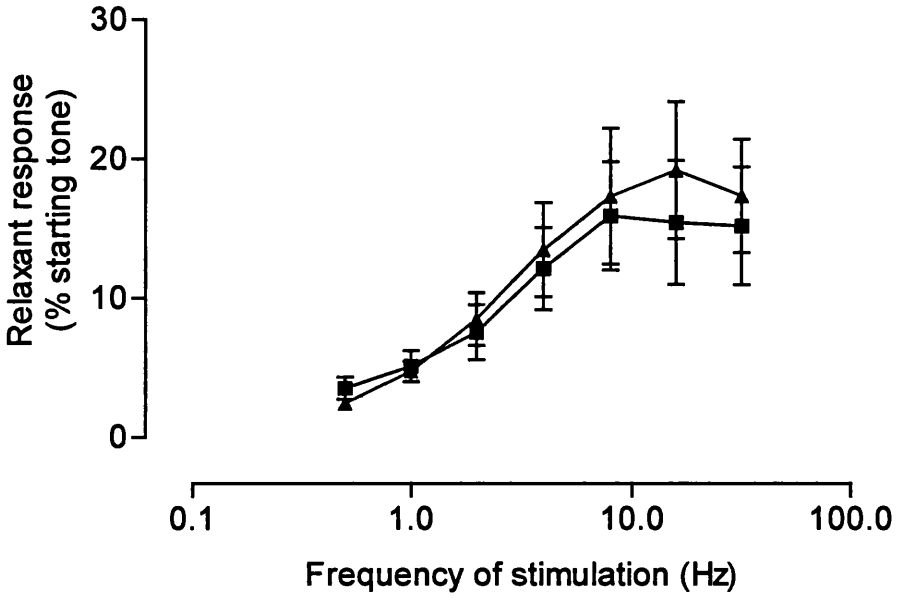


Figure 6.12. Effect of L-NAME ($1x10^{-4}$M) on the EFS-induced (EFS: 0.3 ms, 0.5-32 Hz, 60 V, train length 10 s, interval 110 s) relaxant amplitude of the porcine internal anal sphincter. The points represent mean values and the vertical bars s.e.mean.



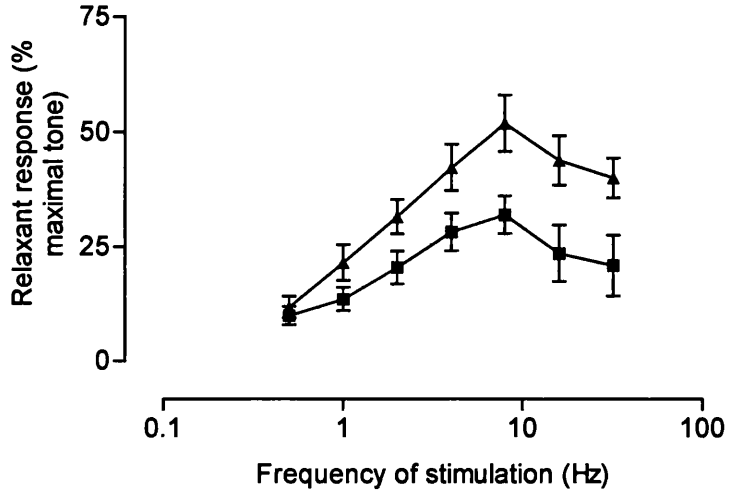
Frequency of stimulation (Hz)	Control (n=9)		+ L-Name (n=4)	
	Mean relaxant amplitude	(s.e.mean)	Mean relaxant amplitude	(s.e.mean)
0.5	0.2	0.1	0.1	0.0
1.0	0.2	0.1	0.2	0.0
2.0	0.4	0.2	0.2	0.0
4.0	0.6	0.3	0.2	0.0
8.0	0.8	0.3	0.3	0.1
16.0	1.0	0.3	0.3	0.1
32.0	0.9	0.3	0.4	0.1

Figure 6.13. Effect of suramin ($3X10^{-4}$ M) on the relaxant response of porcine internal anal sphincter tissue (IAS) due to EFS (EFS: 0.3 ms, 0.5-32 Hz, 60 V, train length 10 s, interval 110 s) (n=5). There was no significant change in the relaxation response for the control preparation (■) compared with suramin (▲) for each of the frequencies examined *(P = NS, paired t test)*.



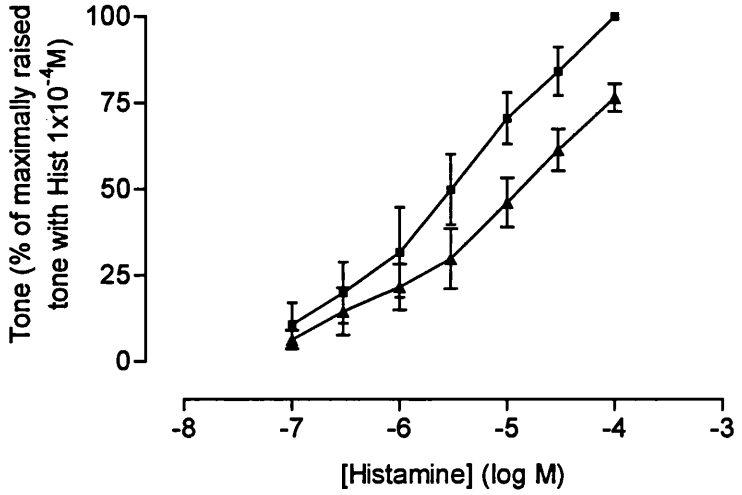
Frequency of stimulation (Hz)	Control (n=5)		+ Suramin (n=5)	
	Relaxant response (% starting tone)	(s.e.mean)	Relaxant response (% starting tone)	(s.e.mean)
0.5	3.5	0.8	2.5	0.4
1.0	5.1	1.1	4.8	0.7
2.0	7.6	2.0	8.5	1.9
4.0	12.1	3.0	13.5	3.4
8.0	15.9	3.9	17.3	4.9
16.0	15.5	4.4	19.2	4.9
32.0	15.2	4.2	17.4	4.1

Figure 6.14. Effect of prazosin ($1x10^{-7}$ M) on the relaxant response of porcine internal anal sphincter tissue (IAS) due to EFS (EFS: 0.3 ms, 0.5-32 Hz, 60 V, train length 10 s, interval 110 s) (n=7). There was a significant increase in the relaxation response with prazosin (▲) as compared with the control preparation (■) for the frequencies above 0.5 Hz *(P < 0.05, paired t test)*.



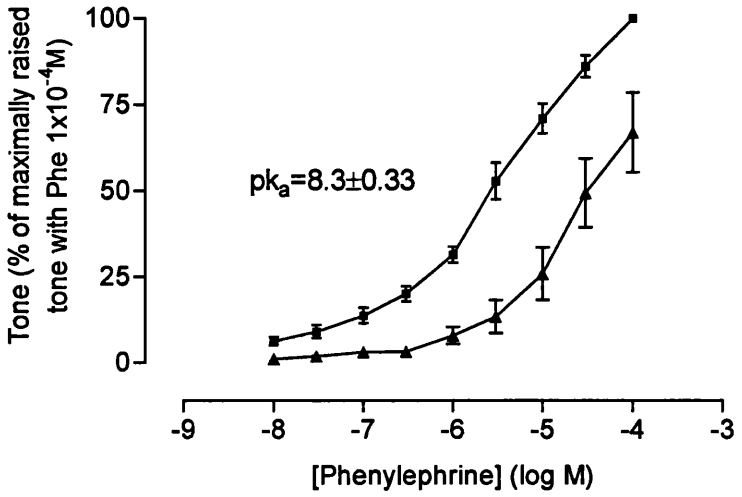
Frequency of stimulation (Hz)	Control (n=6)		+ Prazosin (n=6)	
	Relaxant response (% starting tone)	(s.e.mean)	Relaxant response (% starting tone)	(s.e.mean)
0.5	9.9	2.0	11.8	2.4
1.0	13.5	2.5	21.5	3.9
2.0	20.4	3.6	31.5	3.7
4.0	28.2	4.1	42.2	5.0
8.0	31.9	4.1	51.9	6.1
16.0	23.5	6.1	43.8	5.3
32.0	20.9	6.6	40.0	4.3

Figure 6.15. Effect of prazosin ($1x10^{-6}$ M) (▲) on the histamine (■) dose-response curve of porcine internal anal sphincter (IAS) (n=3). The dose-response curve is shifted to the right indicating a reduction of the contractile effect of histamine.



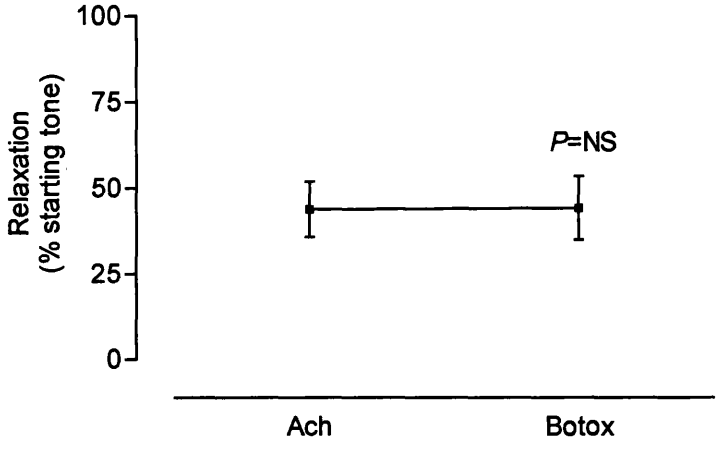
[Histamine] M	Number of muscle strips	Contraction (% maximum)	
		Control	+ Prazosin
$1x10^{-7}$	3	10.7	6.3
$3x10^{-7}$	3	20.0	14.5
$1x10^{-6}$	3	31.7	21.6
$3x10^{-6}$	3	49.9	29.8
$1x10^{-5}$	3	70.6	46.1
$3x10^{-5}$	3	84.2	61.4
$1x10^{-4}$	3	100.0	76.6

Figure 6.16. Effect of the α_1-antagonist indoramin ($1x10^{-7}$ M) (▲) on the phenylephrine dose-response curve (■) in porcine internal anal sphincter (IAS). The pK_a = 8.3±0.33, which is agreement for the pK_a in other tissues. This indicates that phenylephrine-induced contraction of the IAS is mediated through α_1-adrenoceptors.



[Phenylephrine] M	Number of muscle strips	Contraction (% maximum)	
		Control	+ Indoramin
$1x10^{-8}$	7	6.3	1.0
$3x10^{-8}$	7	9.1	1.9
$1x10^{-7}$	7	13.8	3.1
$3x10^{-7}$	7	20.1	3.3
$1x10^{-6}$	7	31.4	7.9
$3x10^{-6}$	7	52.9	13.5
$1x10^{-5}$	7	71.0	25.9
$3x10^{-5}$	7	86.2	49.4
$1x10^{-4}$	7	100.0	67.0

Figure 6.17. Effect of one-hour incubation of one unit of botulinum toxin (Botox®) on the acetylcholine (1x10-3M) induced relaxation of porcine internal anal sphincter (IAS). There is no difference in the relaxant response seen after incubation with botulinum toxin *(P = NS, paired t test).*



	Number of muscle strips	Relaxation (% basal tone)	
		mean	s.e.mean
Ach	5	43.9	8.1
Ach + Botox	5	44.3	9.2

CHAPTER SEVEN

RELATIONSHIP BETWEEN INTERNAL ANAL SPHINCTER TONE AND NEUROGENICALLY OR PHARMACOLOGICALLY MEDIATED RELAXATION

7.1. INTRODUCTION

From the observations by Nothmann and Schuster (1974), Hancock (1977), Lin (1989), and Farouk *et al.* (1994) it is generally accepted that most patients with chronic anal fissures have a higher MRP than normal subjects believed to be hypertonia of the IAS. Whilst lateral internal anal sphincterotomy results in a high healing rate for chronic anal fissures Boulos and Araujo (1984) demonstrated a sustained reduction in the MRP. This has the disadvantage that in the long term this reduction of MRP may lead to disturbances in continence as reported by Khubchandani and Reed (1989). Topical agents, namely GTN and Diltiazem, heal anal fissures with a transient reduction of MRP. Lund and Scholefield (1997) and Carapeti *et al.* (1999) showed fissures in two-thirds of patients heal, although the healing rates are lower in recent studies by Pitt *et al.* (1999), Jonas *et al.* (1999) and Altomare *et al.* (2000). There is a group of patients in whom the fissures do not heal despite experiencing an adequate reduction of the MRP with treatment. This was demonstrated in chapter three where 14 (33%) of 33 patients with chronic anal fissures failed to heal with topical diltiazem after a two month course despite a significant reduction in MRP. The reasons for failure of some anal fissures with high internal anal sphincter tones despite significant reduction in MRP is unclear.

There is evidence to suggest that the physiological behaviour of the IAS in anal fissures is altered. Hancock (1977) demonstrated the presence of ultraslow waves in 10 of 12 patients with chronic anal fissures, compared with 2 of 40 control subjects. Similarly Schouten and Blankensteijn (1992) observed these waves in 29 of 58 patients with

fissures as compared with 2 of 20 control subjects. Ojo-Aromokudo *et al.* (1998) demonstrated an up regulated β adrenoceptor response to the β_2-agonist salbutamol in patients with fissures compared with controls as compared with relaxant effects of the α_1-antagonist indoramin. Regadas *et al.* (1993) compared the responses to the α-adrenoceptor agonist phenylephrine and the β_2-agonist isoproterenol of the IAS from 7 patients with chronic anal fissures and increased anal canal pressures and in 5 control patients with haemorrhoids and normal resting anal canal pressures. The sensitivity to phenylephrine in both groups was similar but a significant difference in sensitivity in the experimental group undergoing relaxation using isoproterenol. Though this up regulation has not been validated in the work presented in this thesis an explanation for altered physiology of the IAS in fissures needs clarification.

Jonas *et al.* (2001c) investigated in an *in vitro* setting with isolated sheep internal anal sphincter whether GTN and diltiazem have an additive effect. Maximal concentrations of GTN and diltiazem each decreased sheep IAS tone by approximately 70%. The combined effect of GTN and diltiazem was complete abolition of IAS tone irrespective of the order in which they were added.

The aim of this study was to investigate the effect of EFS, GTN and diltiazem on an increasing tone of porcine IAS muscle strips in order to explain why some pharmacological agents may not heal anal fissures associated with high IAS tone.

7.2. METHODS

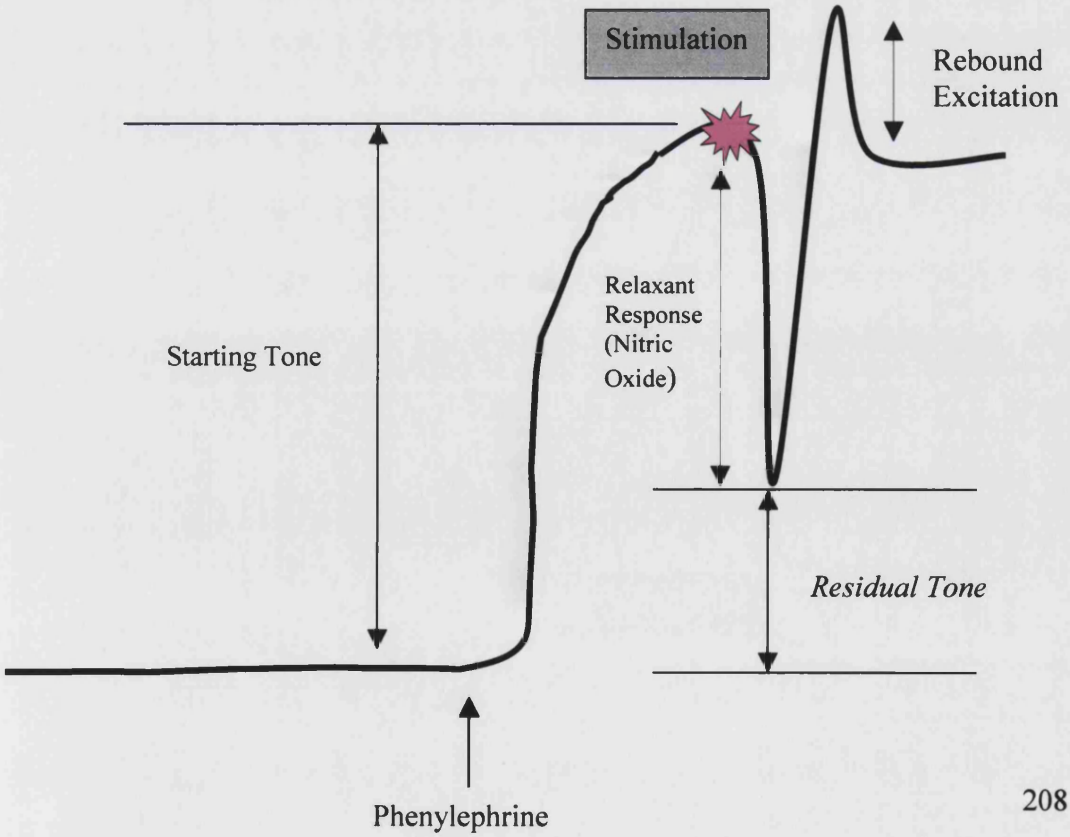
Female Landrace pigs (live weights 80-120kg) were killed at the abbatoir in accordance with approved methods, and the sphincter muscles complexes were removed en bloc and transported in modified Kreb's solution to the laboratory where the IAS tissue was dissected free.

Muscle strips of IAS (guinea-pig: 4mm x 1mm, porcine 5-10mm x 2mm) were suspended in organ-baths containing modified Krebs' solution. One end of the preparation was attached to a rigid support and the other, using silk thread, to an isometric force-displacement transducer (Biopac TSD 125 C). The data were presented with "Acquire software (Acqknowledge 2.0)" computer software, through a MPW100 data acquisition system (Biopac). The porcine muscle strips had an initial load of 2 g applied, and after equilibration (45 min), phenylephrine was added in increasing doses to provide a bath-concentration from $1x10^{-6}$M - $1x10^{-3}$M.

Electrical field stimuli (EFS) was applied at the maximal frequency of 8-16 Hz, using 0.3 ms biphasic pulses at supramaximal voltage for 30 s, and induced maximum relaxations of 0.7 ± 0.13 g. After electrical field stimulation (EFS) of the muscle strips at a specified frequency the tissue responses were allowed to stabilise before the relaxant responses of the contracted porcine strips to GTN ($2.2x10^{-4}$M, $6.6x10^{-4}$M, $2.2x10^{-3}$M) and diltiazem ($1x10^{-4}$M) were measured.

The relaxant responses for EFS, GTN and diltiazem were measured as tone (as a percentage of maximal tone) vs. residual tone (as a percentage of maximal tone). The "maximal tone" is the end tone achieved after cumulative addition of phenylephrine to the highest concentration used ($1x10^{-3}M$). The "residual tone" is the tone achieved at the point of maximal relaxation after the application of EFS, GTN or diltiazem (*fig.*7.1.).

Figure 7.1. Diagrammatic representation of the response of isolated porcine internal anal sphincter (IAS) tissue suspended in oxygenated modified Kreb's solution to electrical field stimulation (EFS: 0.3 ms, 0.5-32 Hz, 60 V, train length 10 s, interval 110 s). Note the contraction of the tissue in response to phenylephrine and the subsequent starting tone prior to stimulation. After stimulation a relaxant response is produced with an ensuing rebound excitation. The residual tone refers to the tone within the preparation at the end of the relaxant response.



7.3. RESULTS FOR PORCINE IAS MUSCLE STRIPS

Electrical Field Stimulation

The porcine muscle strips had an initial load of 2 g applied, and after equilibration (45 min), by which time the tone had dropped to 1.7 ± 0.2 g (n = 9, mean ± s.e. mean), phenylephrine was added in increasing doses to provide bath-concentration from $1x10^{-6}$M - $1x10^{-3}$M. As tone in the porcine IAS was raised by phenylephrine ($1x10^{-6}$M - $1x10^{-3}$M), the relaxant response to the same frequency of EFS (8 Hz or 16 Hz) increased *(fig.7.2).* The regression of this relationship was exponential *(r^2=0.16, d.f.=118) (fig.7.3.).* The results for 10 muscle strips are shown in *Appendix 37.* Phenylephrine evoked concentration-dependent sustained contractions that reached a clear maximum level (4.8 ± 0.9 g) within this range. The residual tone, i.e. the level of tone to which the preparation relaxed during EFS, was proportional to the level of tone at which EFS was applied *(fig.7.4.).* The results for 10 muscle strips are shown in *Appendix 38.* The regression of residual tone on level of tone was linear and showed a statistically significant correlation *(r^2 = 0.90, P << 0.001, d.f. = 120).*

Figure 7.2. The increase in the relaxant response in porcine internal anal sphincter (IAS) as the starting tone of the tissue prior to electrical field stimulation is increased (EFS: 0.3 ms, 8 Hz, 60 V, train length 10 s, interval 110 s).

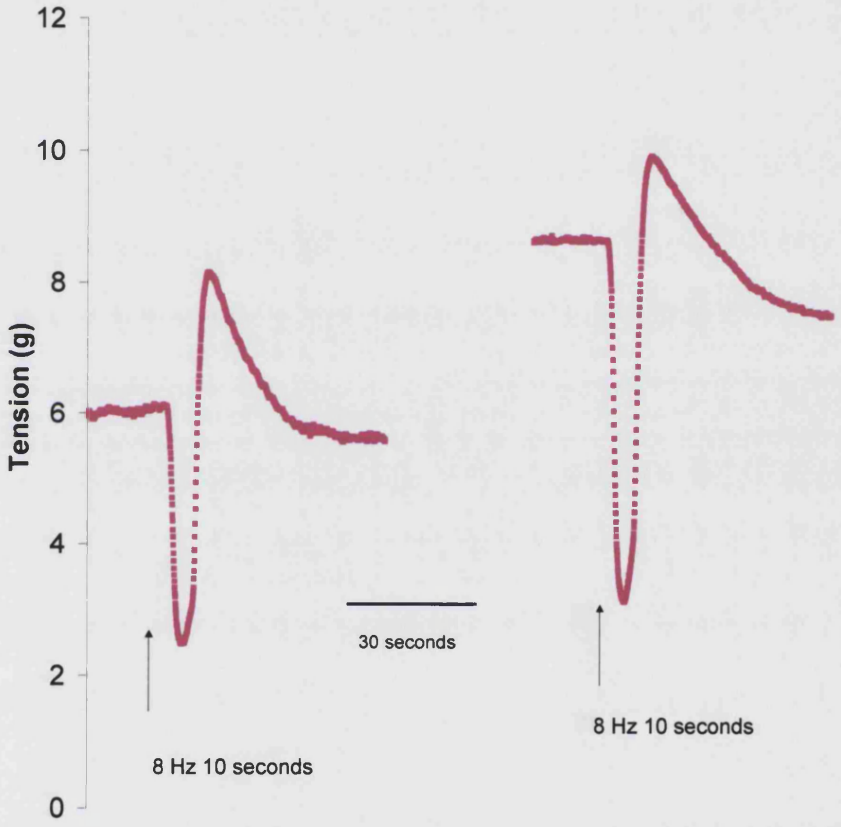


Figure 7.3. Increase of the relaxant response of porcine internal anal sphincter (IAS) to electrical field stimulation (EFS: 0.3 ms, 16 Hz, 60 V, train length 10 s, interval 110 s) on tone raised by phenylephrine (1 μM-1 mM). There is an expontential relationship between the two variables (r^2=0.16, d.f.=118).

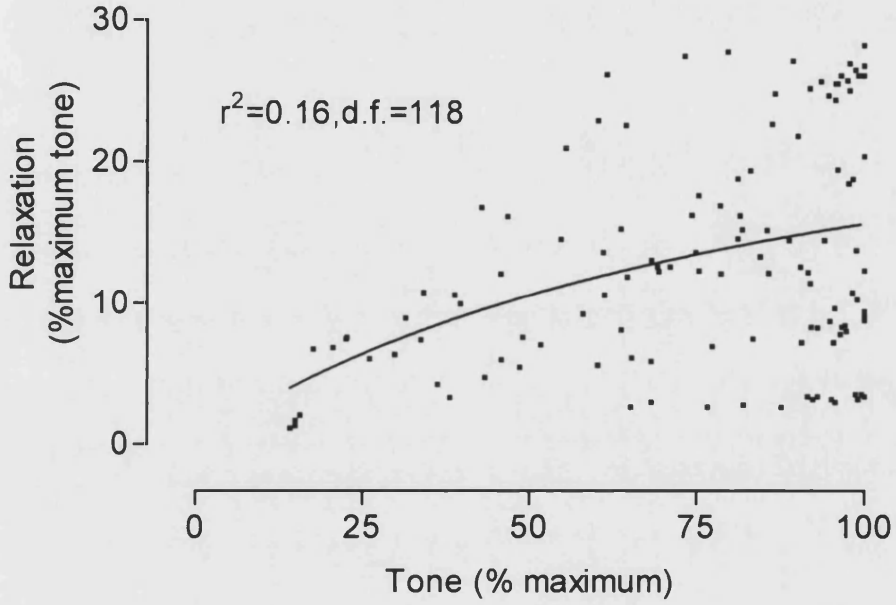
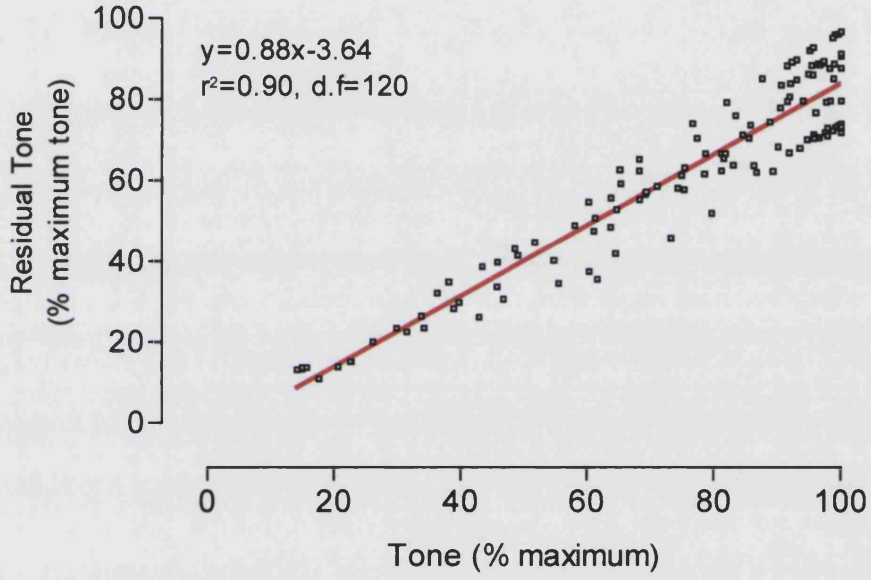


Figure 7.4. Residual tone of response to electrical field stimulation (EFS: 0.3 ms, 16 Hz, 60 V, train length 10 s, interval 110 s) on tone raised by phenylephrine (1 μM-1 mM) in the porcine internal anal sphincter (IAS). There is statistically significant linear relationship between starting tone and residual tone after EFS (y=0.88x-3.64, r^2=0.90, d.f.=120). These are the same data as represented in figure 7.3.



Glyceryl Trinitrate

The results for 21 muscle strips are shown in *Appendix 39*. The relaxant responses to GTN in different concentrations ($2.2x10^{-4}$M, $6.6x10^{-4}$M, $2.2x10^{-3}$M) were quantified. An initial load of 2 g was applied, and after equilibration (45 min), by which time the tone had dropped to 1.3 ± 0.3 g (n = 8, mean ± s.e. mean), phenylephrine was added in increasing doses to provide bath-concentrations from $1x10^{-6}$M - $1x10^{-3}$M. Phenylephrine evoked concentration-dependent sustained contractions that reached a clear maximum level (4.51 ± 0.9 g) within this range. For each of the concentrations of GTN the residual tone, i.e. the level of tone to which the preparation relaxed during GTN, was proportional to the level of tone at which GTN was applied. For this the regression of residual tone on level of tone was linear and again showed a highly statistically significant correlation *(fig.7.5., fig.7.6., fig.7.7.)*.

Figure 7.5. Relationship of tone (% maximum) to residual tone (% maximum) for porcine internal anal sphincter (IAS) relaxation induced by glyceryl trinitrate (GTN: $2.2x10^{-4}$ M). There is a linear relationship between the starting tone and residual tone after addition of GTN y=1.04x-10.96, r^2=0.95, d.f.=21).

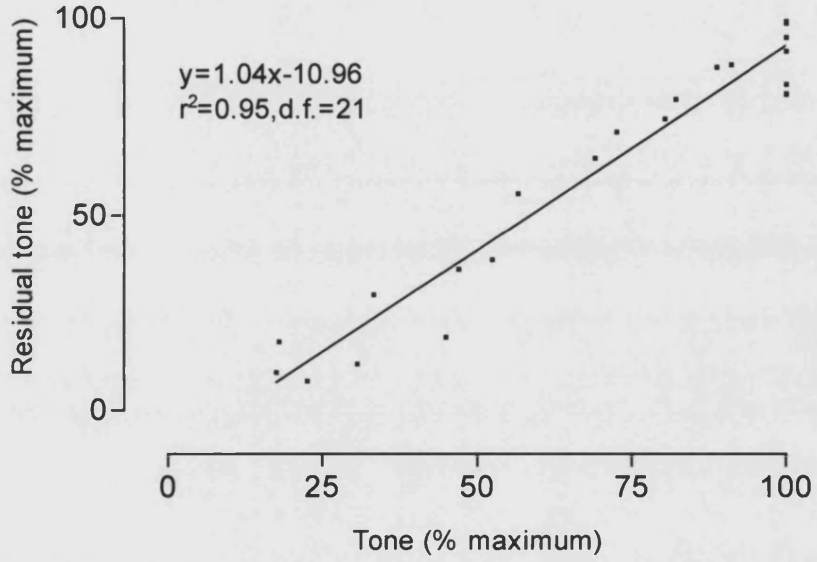


Figure 7.6. Relationship of tone (% maximum) to residual tone (% maximum) for porcine internal anal sphincter (IAS) relaxation induced by glyceryl trinitrate (GTN: $6.6x10^{-4}$ M). There is a linear relationship between the starting tone and residual tone after addition of GTN y=0.92x-11.01, r^2=0.87, d.f.=20).

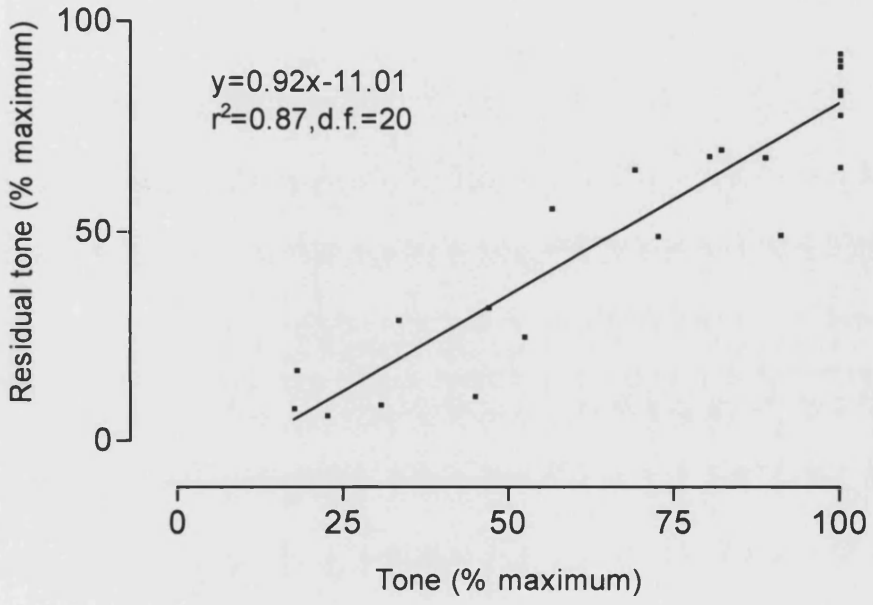
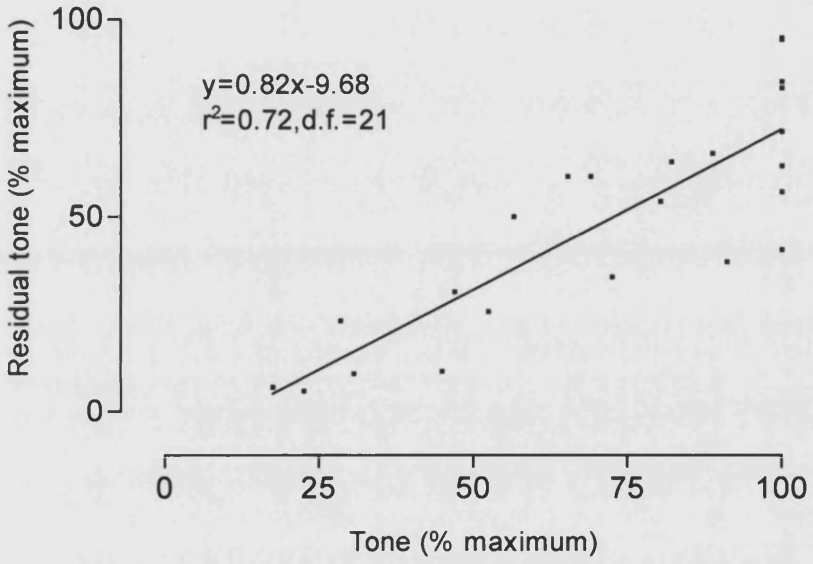


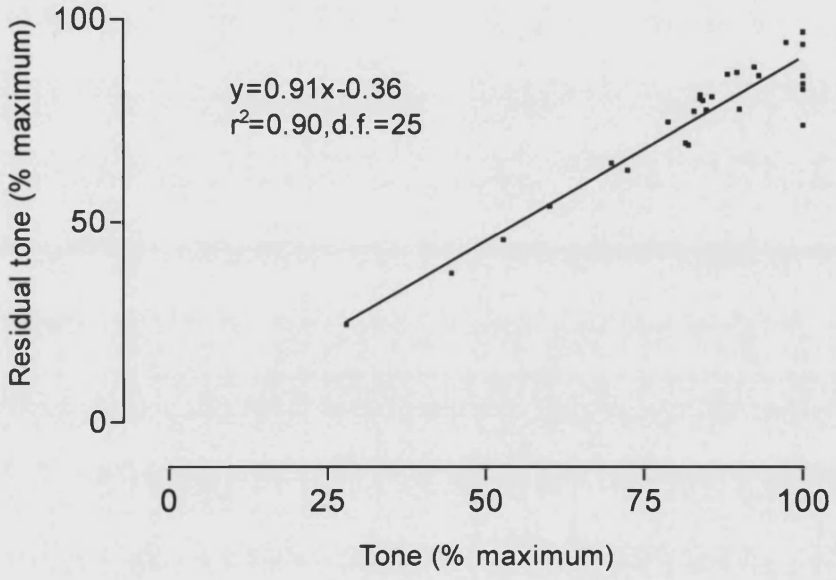
Figure 7.7. Relationship of tone (% maximum) to residual tone (% maximum) for porcine internal anal sphincter (IAS) relaxation induced by glyceryl trinitrate (GTN: $2.2x10^{-3}$ M). There is a linear relationship between the starting tone and residual tone after addition of GTN y=0.72x-9.68, r^2=0.72, d.f.=20).



DILTIAZEM

The results for 6 muscle strips are shown in *Appendix 40.* An initial load of 2 g was applied, and after equilibration (45 min), by which time the tone was 2.2 ± 0.3 g (n = 7, mean ± s.e. mean), phenylephrine was added in increasing doses to provide a bath-concentration from $1x10^{-6}$M - $1x10^{-3}$M. Phenylephrine evoked concentration-dependent sustained contractions that reached a clear maximum level (2.8 ± 0.6 g) within this range. For diltiazem ($1x10^{-4}$ M) the residual tone, i.e. the level of tone to which the preparation relaxed during diltiazem, was proportional to the level of tone at which diltiazem was applied. For this the regression of residual tone on level of tone was linear and again showed a statistically significant correlation *(fig.7.8.).*

Figure 7.8. Relationship of tone (% maximum) to residual tone (% maximum) for porcine internal anal sphincter (IAS) relaxation induced by diltiazem ($1x10^{-4}$ M). There is a linear relationship between the starting tone and residual tone after addition of diltiazem ($y=0.91x-0.36$, $r^2=0.90$, d.f.=25).



7.4. DISCUSSION

This discussion relates the relaxant responses of contracted isolated IAS muscle strips to EFS, GTN and diltiazem and proposes a possible theory for failure of some chronic anal fissures with sphincter hypertonia to respond to topical pharmacological therapy despite significant reduction in resting anal canal pressure. In order to extrapolate the pharmacological model to the clinical situation similarities are drawn between the *in vitro* and *in vivo* setting. These are based on evidence that neurally mediated relaxation of the IAS *in vitro*, through EFS, and *in vivo* with GTN, is modulated through release of nitric oxide; GTN being a nitric oxide donor. The effect of the relaxant response to diltiazem is described separately. Schouten *et al.* (1994) identified the concept of the "ischaemic threshold" which is the IAS muscle tone above which the anoderm is inadequately perfused, and within this "ischaemic zone" chronic anal fissures do not heal. It is proposed the ischaemic threshold varies between individuals.

A hypertonic IAS is reproduced *in vitro* by cumulative addition of the α-adrenoceptor agonist phenylephrine. The "maximal tone" is the end tone achieved after the addition of increasing doses of phenylephrine. The "residual tone" is the tone achieved at the point of maximal relaxation after the application of EFS, GTN or diltiazem (*fig 7.1.*).

The residual tone after EFS, which is the tone achieved from activation of the enteric nerves, also increases as the basal tone of the tissues is increased by phenylephrine, and

followed a linear pattern (*figs.7.2. & 7.3.)*. This indicates at very high tones the resultant residual tone achieved is also high.

Similar observations are made with the three concentrations of GTN and diltiazem. There is a linear relationship between the starting tone and resultant residual tone achieved after relaxation. The importance of the linear relationship of starting tone to the residual tone after either EFS or pharmacological relaxation is relevant to the vascular pathogenesis of anal fissures (Schouten *et al.*, 1996a). Schouten *et al.* (1996b) have demonstrated that the perfusion of the posterior commissure of the anal canal, where the majority of idiopathic chronic anal fissures occur, is strongly related to the anal canal pressure. There is an inverse relationship between the maximal anal canal pressure and the flux (vascular perfusion) of the anal canal. Current hypotheses indicate that chronic anal fissures do not heal because of the persistent vascular ischaemia of the fissure [Lund and Scholefield, 1996]. Therefore reduction of the anal canal pressure below a critical level will allow the fissure to heal.

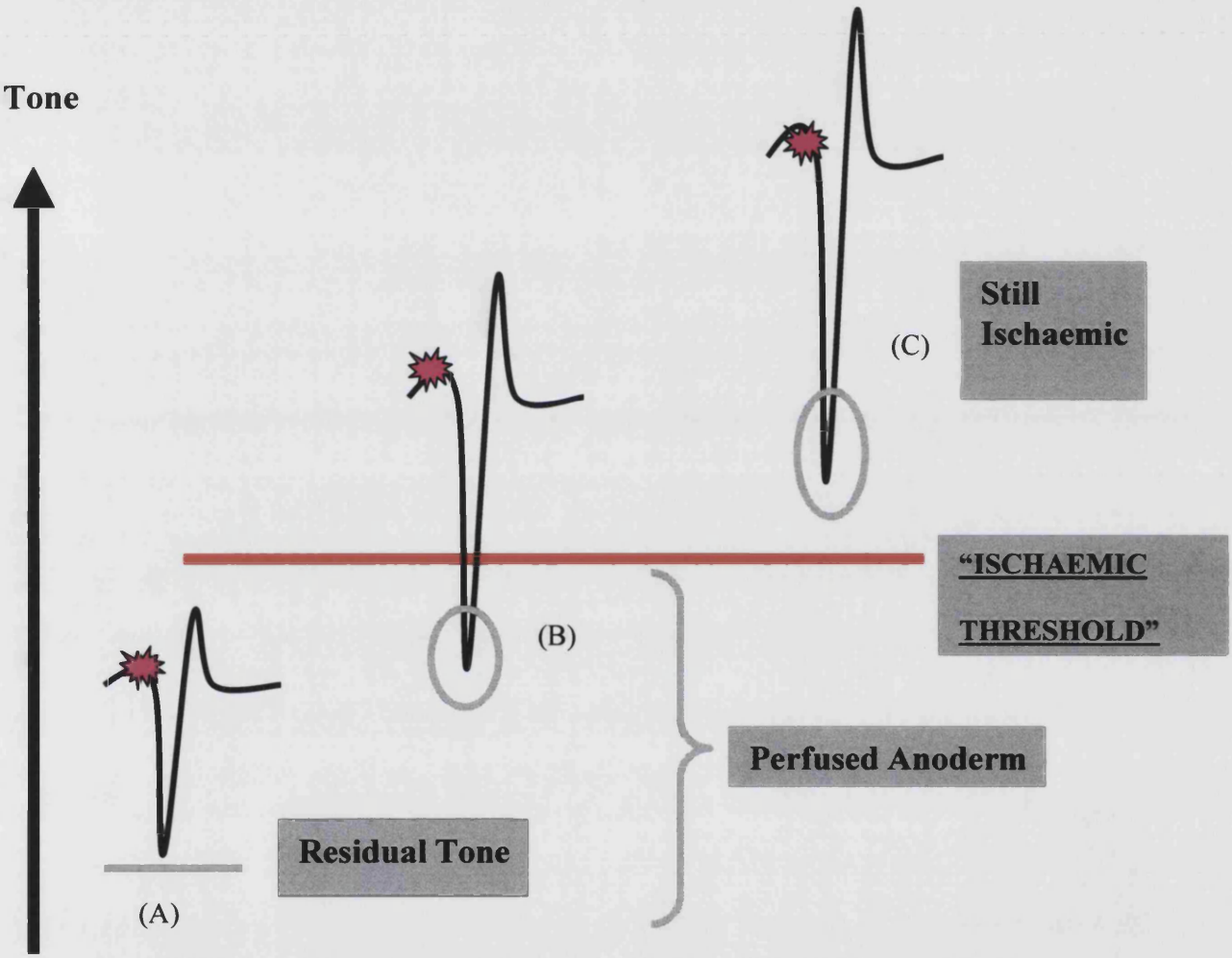
The results presented in this chapter show as the tone of the IAS is increased the relaxant response increases (see *fig. 7.9.*). There is a point above which the tone of the IAS (which is translated clinically into the maximal anal canal pressure) means that the anal canal is not adequately vascularly perfused. This is termed the "ischaemic threshold". The residual tone achieved after EFS above this point may or may not result in perfusion of the anal canal. Points A, B, C in *fig. 7.9.* are explained as follows:

(A) This starting tone of the muscle strip and resultant residual tone achieved after EFS both lie below the "threshold level" for adequate perfusion. Relating this to the clinical situation means that fissures with low pre-treatment MRP and the resultant relaxant stimuli applied will mean that the anoderm is perfused throughout.

(B) This starting tone of the muscle strip lies above the "ischaemic threshold" but resultant residual tone achieved after EFS lies below the "threshold level" for adequate perfusion. Relating this to the clinical situation means that fissures with high pre-treatment MRP may have an inadequately perfused anoderm but after application of relaxant stimuli the anoderm is perfused. Fissures that display this physiological response will heal.

(C) The starting tone here is very high, above the "ischaemic threshold" (clinically analogous to some chronic anal fissures associated with higher resting anal canal pressures). As such they lie in the zone where the anoderm is inadequately perfused. Even with EFS (nitric oxide transmission) the residual tone is not sufficiently low to provide an adequate perfusion of the anal canal. Therefore using nitric oxide donors will not result in an adequate reduction of tone to allow adequate perfusion of the anal canal and fissures that experience this behaviour will not heal. Note also as the starting tone is increased the amplitude of the relaxant response increases.

Figure 7.9. Representation of the relationship increasing tone on of the result of EFS (and indirectly pharmacological relaxation) on the response of isolated IAS muscle strips and its relevance to the "ischaemic" threshold.



It would be ideal if one could relate the MRP of the anal canal and suggest whether a particular dose of an agent will cause healing.

Consider the "ischaemic threshold" which has been defined as the point at which anal canal pressure just allows for adequate perfusion of the anal canal. Above this the anal canal pressure is associated with chronic anal fissures *(fig. 7.10.)*.

Let "Z", an arbitrary figure be termed the "ischaemic index" indicated by the equation:

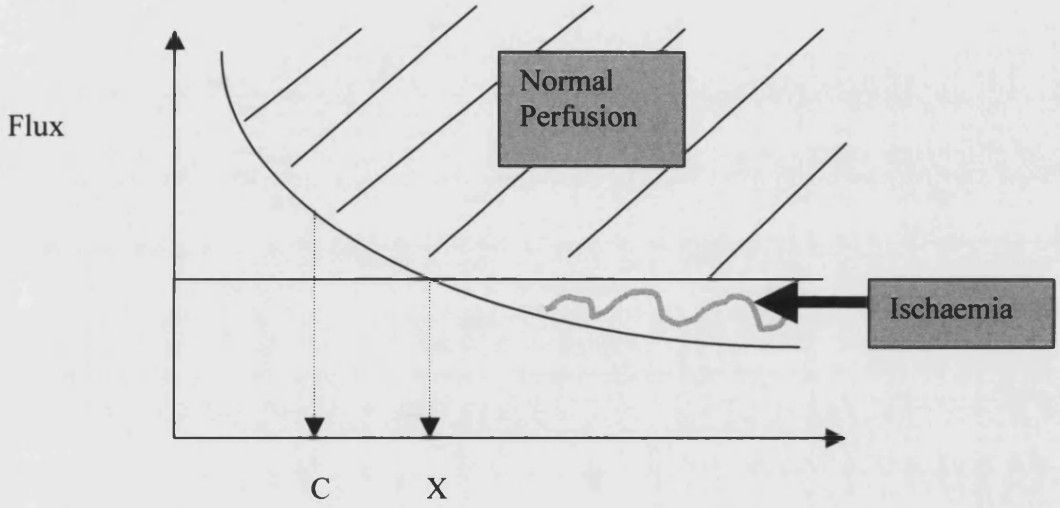
$$Z = X / Y \qquad [1]$$

Where:

X = the maximal anal canal pressure for adequate perfusion of the anal canal.

Y= the anal canal pressure associated with chronic anal fissures, which is assumed to be when the smooth muscles of the internal anal sphincter are maximally contracted.

Figure 7.10. Diagram demonstrating the relationship between anal canal pressure and anodermal blood flow (flux) (Schouten *et al.*, 1994). According to Schouten's hypothesis of the importance of ischaemia in the development of chronic anal fissures there is an anal canal pressure (as indicated by "X") above which the anal canal will not be adequately perfused and a chronic anal fissure may not heal. The point "C" indicates the mean MRP in healthy volunteers.



We can calculate the value of Z from Schouten *et al.'s* (1994) demonstration from 9 patients with chronic anal fissures. Their the mean maximal anal canal pressure was 125 ± 26 mmHg, and that in 31 control subjects was 66 ± 19 mmHg. For this group the ischaemic Z, the "ischaemic index" is calculated by the equation:

$$Z = [\text{MRP in control subjects} + \text{s.d. (mean)}] / \text{MRP in patients with fissures}$$

$$= (66+19) / 125$$

$$= 0.7 \text{ (to one decimal place)}$$

Thus if the IAS in patients with chronic anal fissures at maximal tone the residual tone (% maximally contracted tone) above which the tissue are not adequately perfused is Z x100 % = 70 %.

Figures 7.11 & 7.12. depicts the starting tone ,T, above which the relaxation of the tissues by either EFS of pharmacological intervention will not be adequate for perfusion of the anal canal.

Figure 7.11. The concept of the "ischaemic index", "Z", the figure beyond which the anal canal is inadequately perfused "X" which is the relationship demonstrated in the following equation:

$$Z = X / Y$$

X = the maximal anal canal pressure for adequate perfusion of the anal canal.

Y= the anal canal pressure associated with chronic anal fissures, which is assumed to be when the smooth muscles of the internal anal sphincter are maximally contracted.

"C" is the anal canal pressure associated with healthy volunteers.

"T" indicates the MRP above which relaxation of the muscle through either neural stimulation or pharmacological manipulation will not reduce the MRP to below point "X".

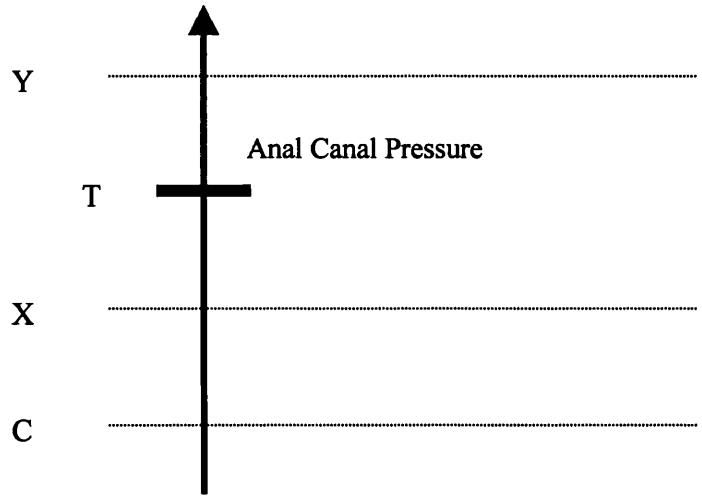
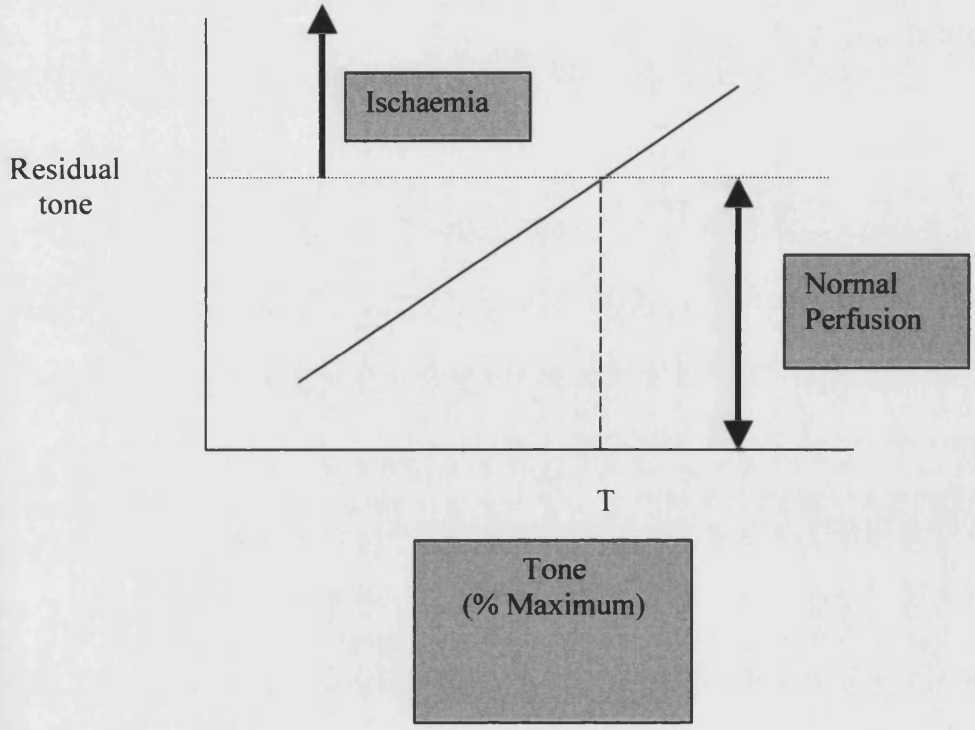


Figure 7.12. Diagram representing the relationship between the residual tone of the internal anal sphincter after relaxation (as a fraction of the maximal tone), the staring tone (as a percentage of maximal tone) prior to this relaxation, and the concept of ischaemia of the anal canal.



"T" is the tone of the smooth muscle, as a percentage of the maximal tone achieved by contraction, above which relaxation of the smooth muscle for the value interpolated by "Z" relates to inadequate perfusion of the anal canal.

Because of the linear relationship between the starting tone and residual tone, for the EFS frequency denoted and the drug concentrations used, T can be calculated.

For example for EFS (16Hz) the regression equation is:

$$y = 0.88\,x - 3.64$$

If $y = 70$, $x = T$

$$T = (70 + 3.64) / 0.88$$

$$T = 84$$

Therefore if the IAS is contracted to beyond 84 % of its maximal contacted tone, the stimulation of the enteric nerves will not be enough to allow adequate vascular perfusion of the anal canal.

Application of these results for the pharmacological agents used shows varying values for T :

	T (The % of maximally contracted tone Above this % use of the [drug] stated will be Ineffective in reducing the tone to below X (see *fig.7.11*)
GTN (2.2×10^{-4} M),	T = 78 %
GTN (6.6×10^{-4} M)	T = 88 %
GTN (2.2×10^{-3} M)	T = 97 %
Diltiazem (1×10^{-4} M)	T = 77 %

These results indicate that if the ischaemic index is equivalent to a residual tone of 70 %, one can interpolate the IAS tone at which GTN becomes ineffective. The results

demonstrate there is concentration dependence for GTN, with higher concentrations proving possibly more effective. If one takes into account of the amount and concentration of GTN in biophase following topical application the reduction noted might not be great enough to allow vascular perfusion in any case, therefore the effect of concentration may not be important.

In conclusion these results would suggest that in the clinical setting those patients with chronic anal fissures in whom GTN and diltiazem reduces the MRP significantly but still fail to heal may be related to the failure of these agents to reduce the MRP *sufficiently* to allow adequate perfusion of the anoderm. The amplitude of the relaxation is dependent on the starting MRP and may be dose dependent. This chapter has provided direct evidence in the *in vitro* setting for this hypothesis.

CONCLUSION

The evolution of alternative therapeutic strategies over the last decade has enabled clinicians to treat chronic anal fissures more effectively. The increasing interest in conservative therapies has arisen because of concerns over the long term functional effects of surgical division or dilatation of the IAS.

The management of chronic anal fissures was investigated by Karandikhar *et al.* (2002) in a postal survey. Among 357 (78%) responders of 452 members of the Association of Coloproctology of Great Britain and Ireland, 70% regarded GTN as the treatment of choice. Fissures that did not heal after 6-12 weeks of treatment were deemed as a failure. The extent of surgical division of the IAS varied, as 42% divided the entire length of the IAS. Anal dilatation was selectively practiced by 37.6%. The authors then explored the treatments offered for a 40 year old continent male and continent postpartum female. In the male patient 90.6% would perform lateral sphincterotomy; 5% would perform endoanal ultrasonography, and 3.6% would perform anorectal physiology. In the female patient 76.6% would perform lateral sphincterotomy, but 89% would evaluate the sphincter preoperatively. 33% by endoanal ultrasound, 30% by anorectal physiology and 26% by both. The results suggest that in patients who fail to respond to conservative treatment lateral sphincterotomy is the surgical procedure of choice and in females preoperative evaluation of the IAS is recommended.

The first-line treatment of chronic anal fissures with topical agents has led to management algorithms that can be effectively employed under the supervision of nurse

specialists. Porrett *et al.* (2002) examined the outcome of 135 patients with chronic anal fissures over a 3-year period. Patients were initially treated with a single course of 0.2% GTN, and those that failed were offered 0.2% GTN again, 0.4% GTN or surgery. Surgical treatment included anoplasty in all females, and in those males with normal or low resting anal canal pressures, and lateral sphincterotomy in males with high anal canal pressures. At a median follow up of 9 months, 66 (49%) healed with resolution of symptoms after a single course of 0.2% GTN (62% acute, 41% chronic fissures). 74% healed after a second course of 0.2% GTN (100% acute, 71% chronic fissures) with no advantage in using 0.4% GTN. In 20 (15%) patients the fissures healed by surgical treatment (10 anoplasty, 10 lateral anal sphincterotomy) and one patient suffered soiling after surgery. This approach was effective in that a clear protocol was followed with good effect.

Brown *et al.* (2002) also used algorithms with particular reference to the management of persistent and recurrent chronic fissures. They highlighted that with GTN as initial treatment, fissures persist in 32-54% of cases [Kennedy *et al.* (1999), Altomare *et al.* (2000), Lund and Scholefield (1997b)]. Furthermore Dorfmann *et al.* (1999) had shown that fissures recurred in upto 63%. Irrespective of the mode of therapy these persistent or recurrent fissures pose a particular challenge.

Failure of GTN therapy may be related to non-compliance due to headaches, or inadequate length of treatment. Pitt *et al.* (1999), Richard *et al.* (2000) and Skinner *et al.* (2001) stated that approximately 20% of patients cease treatment because of side effects.

Salgado and Berman (1999) showed that the use of a slotted applicator tip (Apthorp Pharmacy, New York, USA) reduced many of the side effects experienced from digital application. Whilst Watson *et al.* (1996) suggested that the minimum length of period of treatment with GTN is 4 weeks; there is no consensus over the optimal period of treatment for GTN, as the duration of treatment reported has varied from 4 to 12 weeks.

Whilst botulinum toxin is an effective modality for initial treatment, Brisinda *et al.* (1999) successfully treated 9 patients with fissures that persisted after GTN. Jost *et al.* (1999b) also used a repeat injection of botulinum toxin to treat fissures that persisted. There is evidence of the use of alternative agents that do not heal with initial pharmacological therapy. Jonas *et al.* (2002) showed topical diltiazem heals fissures that failed with GTN therapy and Cook *et al.* (1999a) reported healing in 4 patients with nifedipine who failed treatment with GTN. Most would advocate surgery in those that have failed to heal with repeat pharmacological therapy. Nevertheless Richard *et al.* (2000) showed that in their randomized trial comparing lateral sphincterotomy with GTN, no recurrences occurred following surgery, but 45.4% of 44 patients who were initially treated with GTN subsequently required sphincterotomy. Whilst lateral sphincterotomy is the procedure of choice for the majority, alternative surgical modalities such as anoplasty is advocated in those with lower anal canal pressures.

In those that fail to respond or recur after surgery secondary causes which include Crohn's disease, HIV infection, tuberculous disease or malignancy must be considered before further surgery or pharmacological therapy, particularly when fissures are multiple

or not in the midline. Lewis *et al.* (1988) found that 5 (24%) of 21 patients with persistent fissures following lateral sphincterotomy had Crohn's disease. Sweeney *et al.* (1988) showed that many fissures associated with Crohn's disease heal with conservative treatment alone, though of those that do not approximately 80% will heal with immunosuppressive treatment. Whilst most fissures associated with Crohn's disease are painless, Fleshner *et al.* (1995) still advocated a lateral sphincterotomy. Barrett *et al.* (1998) noted that of 260 patients with anorectal disease 32% had anal fissures. Not all fissures associated with HIV infection are associated with sphincter hypertonia and in these cases sphincterotomy should be used with caution. In the management of the tuberculous fissure Candela *et al.* (1999) and Whalen *et al.* (1980) have reported healing with anti tuberculous therapy.

Nyam *et al.* (1999) showed that conservative management with high fibre and fluid intake will heal upto two-thirds of patients with unhealed idiopathic fissures. Of 11% of fissures that recurred in a series of 549 patents reported by Garcia-Aguilar *et al.* (1996), 30% of these were reoperated on and the remainder were treated successfully with conservative therapy. In a larger series of 2030 patients Argov *et al.* (2000) reported a 1% incidence of recurrent or persistent fissures. Sphincterotomy on the contralateral side in those with high resting anal canal pressures resulted in healing in all patients. Nyam *et al.* (1995) used anal advancement flap successfully in 7 patients whose fissures did not heal or recurred following lateral sphincterotomy.

This thesis has sought to re-explore and clarify issues raised in the literature since the introduction of pharmacological agents almost a decade ago.

The use of topical 2% diltiazem is an effective alternative to topical 0.2% GTN as first line therapy for the treatment of chronic anal fissures without risk of potential headaches and poor compliance. Manometric evaluation confirmed higher MRP in patients with fissures compared with volunteers, and in those with anal spasm. A healing rate of 58% was comparable to that seen with other topical pharmacological agents, and those fissures that heal do so with a significant reduction the MRP. Of note there was also a reduction in the MRP in fissures that did not heal and this may be explained by the vascular pathogenesis of chronic anal fissures due to failure to attain the ischaemic threshold. The results in this thesis imply that it is not possible to use pretreatment MRP as a prognostic indicator to determine success with topical diltiazem as the MRP is similar in responders and non responders. Furthermore it was not possible to use the presence of a sentinel pile and/or anal spasm as a prognostic indicator. All patients that had healed were asymptomatic. After cessation of treatment the MRP in 12 patients initially healed at two months revealed a return to pretreatment MRPs by 4 months, this is the "reversible chemical sphincterotomy". The use of a second course in persistent fissures failed to decrease the MRP in the patients studied at 4 months, yet 6 healed. This healing may be related to the direct effect of topical diltiazem in the vasculature of the anal canal. The response to the application of topical diltiazem may be classified into three groups: patients with high MRP that heal with a reduction of MRP; patients with high MRP that

fail to heal despite a reduction in MRP; and patients with low MRPs in whom the MRP is unaltered with treatment.

The reluctance of clinicians to directly inject botulinum toxin into the anal sphincter complex led to the feasibility studies that used the J-tip needless injection system to deliver the agent. After initial animal studies to determine an optimal angle of penetrance 10 patients with chronic fissures were recruited, treated with 50 units, and evaluated until 12 weeks. Despite the size of the injected molecule it was effective in reducing the MRP at least to 2 months, with an effective reduction in median pain scores, and without a detrimental effect on continence. The poor healing rate of 5 (50%) of 10 may be related to the inadequate dosages used.

Both oral indoramin and oral salbutamol acting on the sympathetic receptors on the IAS smooth muscle cell decrease MRP in both volunteers and patients over the three hours studied. Whilst 3 patients experienced light headedness with indoramin, 9 subjects experienced tremors with salbutamol. Furthermore there was a transient tachycardia experienced in subjects taking salbutamol. It was felt that salbutamol would not be a suitable agent to continue to investigate. The subsequent study conducted, investigation of a topical pharmacological preparation of indoramin at doses ranging from 10mg to 40mg failed to have any superior effect over placebo. This may be related to the paraffin based delivery substance, or the superior local action of the sympathetic drive. The unsuitability of oral indoramin to treat chronic anal fissures was examined by Pitt *et al.*

(2000). In this trial many more patients experience side effects which included fatigue, dizziness, headache, dry mouth, nasal congestion and retrograde ejaculation.

The necessity to validate an animal model of human IAS tissue was born out of O'Kelly *et al.*'s (1992) and Cook *et al.*'s (1999b) investigations to examine the pharmacological responses of porcine IAS. Whilst Mundey *et al.* (2000) has subsequently validated the responses of sheep IAS muscle, it was felt that this needed to be conducted with porcine tissue. Porcine IAS muscle strips maintained their own muscle tone, contracted in response to histamine and phenylephrine. The muscle strips relaxed in response to isoprenaline and glyceryl trinitrate, and acetylcholine, and ATP. The relaxant response to acetylcholine was abolished by L-NAME, and suramin potentiated the relaxant response of ATP. The relaxant responses to electrical field stimulation were reduced with L-NAME, unaffected by suramin, and potentiated by prazosin. Furthermore prazosin reduced the contractile response of histamine, whilst indoramin reduced the contractile response of phenylephrine. These studies confirmed the similarities of the neural properties of porcine and human IAS, and as the tissue was structurally similar it was felt that this tissue could be suitably used for further investigation.

The final series of experiments investigated the reasons for why in some patients a reduction of MRP is not equated with healing of chronic anal fissures. There was a linear relationship between the residual relaxant tone after electrical field stimulation and the starting tone. Similar effects were seen for relaxant responses demonstrated with glyceryl trinitrate and diltiazem. One can conclude from this that in patients with very

high MRPs healing may not be observed as the relaxation of the IAS that is induced may not be sufficient to permit adequate perfusion of the anoderm. This conclusion may explain why 14 of 33 patients treated with topical diltiazem in chapter 3 failed to heal despite demonstrating a significant reduction in their MRP.

This thesis has sought to investigate current controversies in the treatment of chronic anal fissures. Before appropriate treatment can be instituted it is imperative that the nature of the fissure is identified, as related to the history and the examination findings; and the appropriate consequences of treatment should be discussed with the patient. There is much scope for tailoring pharmacological therapy for the management of chronic anal fissures.

APPENDICES

Appendix 1: GENERAL METHODS

CLINICAL PHYSIOLOGICAL WORK

Anorectal physiological equipment

The equipment used to obtain the measurements of the resting pressure of the IAS was a continually water-perfused eight-channel catheter assembly *(Lewis Medical, Hertfortshire and Mui Scientific, Ontario) (figs.1. & 2.).* The measurements of resting anal canal pressure were obtained by advancing the manometry catheter into the anal canal from the external anal verge. At each point the catheter was not advanced until a steady reading had been observed. This method is similar to that used by Arabi *et al.* (1977) who deemed the maximal resting anal canal pressure (MRP) to be the highest mean pressure at any of these sites. This method is different from the mean value of different pressures measured throughout the anal canal, which was used in Hancock's study (1977); the single point manometry which was used in the assessment of salbutamol in a smaller number of subjects (Ojo-Aromokodo *et al.,* 1998), and differs from pull-through manometry which is used where vector volume manometry is routinely employed. The data was presented with "MMS" computer software package (version 7.0), with a NEC Cyrix 686 MX processor. When required pulse and blood pressure were measured by the author using a manual syphgmometer *(Branchburg, New Jersey).*

Drugs and solvents used

The following pharmacological agents were used in the clinical trials involving human subjects:

INDORAMIN	SMITHKLINE BEECHAM PHARMACEUTICALS
SALBUTAMOL	APPROVED PRESCRIPTION SERVICES
BOTULINUM TOXIN	ALLERGAN LTD.
DILTIAZEM	SLA PHARMA

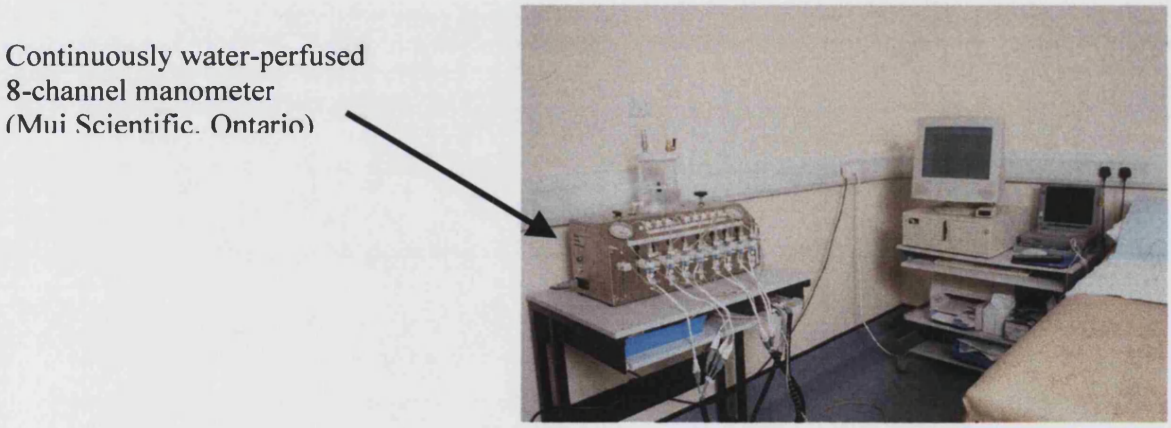


Figure 1. Anorectal physiology suite at the Middlesex Hospital.

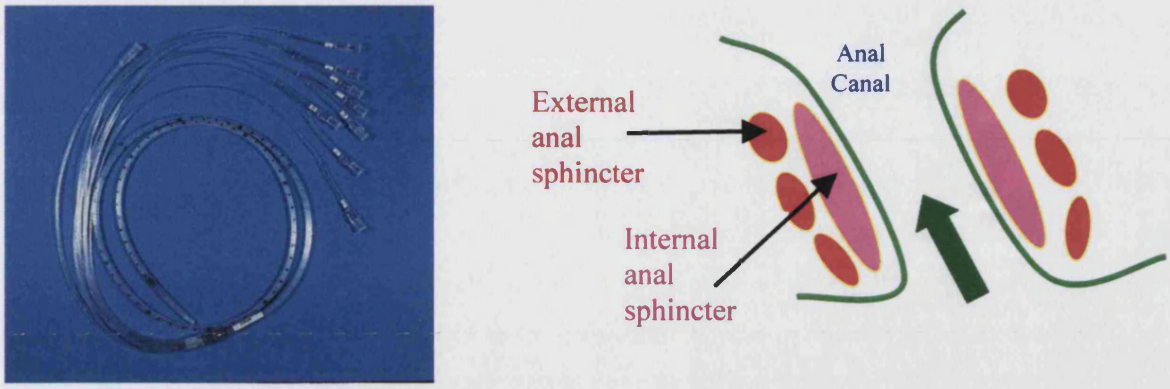


Figure 2. Eight channel catheter assembly and diagram showing advancement of catheter into anal canal

LABORATORY EXPERIMENTAL WORK

Physiological solutions

For all experiments performed on mammalian tissues a modified Krebs' solution was used, of the following compostion (mM): NaCl, 133; KCl, 4.7; NaH_2PO_4, 1.4; $NaHCO_3$, 16.3; $MgSO_4$, 0.6; $CaCl_2$, 2.5; Glucose, 7.7 (Bulbring, 1953). The solution was gassed with 95% O2/ 5% CO2, and after equilibrium the pH was 7.3-7.4.

Animals used

The guinea pigs were male Dunkin Hartley, (Charles River, weight 275-375g). The guinea pigs were sacrificed by cervical dislocation and the pigs were stunned and bled. The pigs were male and female Landrace (live weights 80 –120kg). The pigs were culled in accordance with approved methods.

Organ-bath apparatus

Organ-baths were 5ml or 10ml in volume, side-arm gassed and made of glass *(Linton,Norfolk,UK)*. The baths and reservoirs of the Krebs' solution were maintained at 36.5 ± 0.5°C (unless otherwise stated), and were continually gassed *(fig.3.)*.

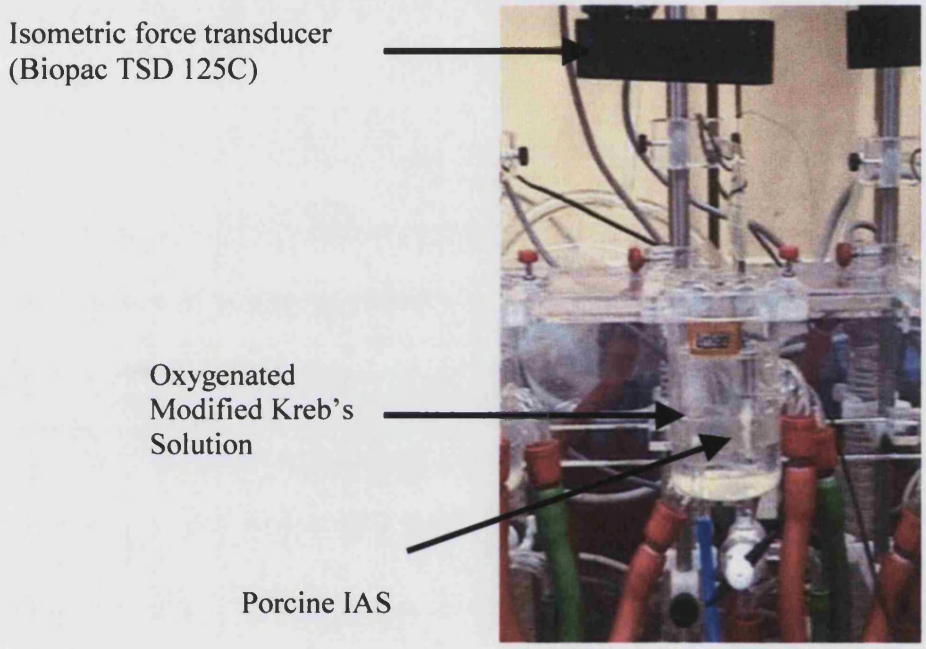


Figure 3. Organ Bath Apparatus

Preparations for organ-bath studies

Guinea-pig internal anal sphincter: The abdomen was opened, internal viscera removed, and the pelvic floor structures were removed. The internal sphincter was dissected free. Porcine internal sphincter: The isolated sphincteric complex was removed from the

abattoir and transported in Kreb's solution. In the laboratory the internal sphincter tissue was dissected free.

Segments of internal anal sphincter (5-10mm x 2mm) were suspended in organ-baths containing Krebs' solution. One end of the preparation was attached to a rigid support and the other, using silk thread, to an isometric force-displacement transducer (Biopac TSD 125 C). The data was presented with "Acquire software (Acqknowledge 2.0)" computer software, through a MPW100 data acquisition system (Biopac). Preparations were preloaded with 0.5-2.0g and were allowed to equilibrate for at least 50 min before any drugs were added. If required the tissues were electrically stimulated (Experimetria S02 stimulator) the following stimulation parameters were used: pulse width (0.3 ms, biphasic); frequency (0.5 - 32 Hz); train length (10 s); interval between trains (110 s) and voltage (supramaximal, 60 V).

Drugs and solvents used

ACETYLCHOLINE CHLORIDE	SIGMA
ADENOSINE 5'-TRIPHOSPHATE (ATP)	SIGMA
ATROPINE SULPHATE	SIGMA
BOTULINUM TOXIN	ALLERGAN LTD.
DILTIAZEM HYDROCHLORIDE	SIGMA

ELOHAES	FRESNIUS
GLYCERYL TRINITRATE (GTN)	FAULDING DBL
S-NITROSOGLUTATHIONE (GSNO)	SIGMA
GUANETHEDINE MONOSULPHATE	SIGMA
HISTAMINE	SIGMA
INDORAMIN HYDROCHLORIDE	SMITHKLINE BEECHAM PHARMACEUTICALS
NORADRENALINE BITARTARATE	SIGMA
N^{W}-NITRO-L-ARGININE METHYL ESTER (L-NAME) HYDROCHLORIDE	SIGMA
METHOXAMINE HYDROCHLORIDE	SIGMA
L-PHENYLEPHRINE HYDROCHLORIDE	SIGMA
PRAZOSIN HYDROCHLORIDE	SIGMA
PROPANOLOL HYDROCHLORIDE	ZENECA PHARMA
SURAMIN (SODIUM SALT)	SIGMA

KREB'S SALTS WERE ALL ANALYTICAL GRADE

Most drugs were made up into stock solutions using distilled water. Noradrenaline was dissolved in ascorbic acid at 0.01 times the molar concentration of noradrenaline.

ANALYSIS OF RESULTS

Statistical analysis of results was carried out using "SPSS" (statistical packages for social sciences) computer software (version 7.5) and "Prism" (version 3.0). Comparison of MRP was conducted after testing for normality. Graphs were drawn using "Prism" version 3.0. A level of probability, if considered in the statistical analysis, of $P \leq 0.05$ was considered significant.

Appendix 2: Maximum Resting Anal Canal Pressure (cmH_2O) in patients analysed over a 6 month period

			START	2 MONTHS	4 MONTHS	6 MONTHS
No.	Sex	Age				
1	Male	**38**	77	83	47	
2	Female	**40**	77	55		53
3	Male	**36**	83	68		
4	Male	**81**	88	75	77	76
5	Female	**35**	88	90		
6	Female	**68**	93	64	100	76
7	Female	**28**	93	75		
8	Female	**29**	93	103	101	125
9	Female	**59**	98	96		
10	Female	**28**	101	79	100	98
11	Female	**27**	101	68		
12	Male	**35**	102	79	117	161
13	Female	**29**	103	61		
14	Male	**40**	108	111	110	134
15	Female	**27**	109	66	51	
16	Male	**37**	110	53	120	137
17	Female	**35**	110	107	134	
18	Male	**27**	110	83		
19	Female	**30**	112	69	88	
20	Male	**28**	112	94		
21	Female	**42**	115	119	132	120
22	Female	**28**	124	123	96	137
23	Male	**33**	128	56	64	113
24	Female	**39**	129	79	135	120
25	Male	**28**	131	81	115	125
26	Female	**27**	137	120		
27	Male	**33**	141	109		
28	Male	**31**	142	124		
19	Male	**29**	143	125	134	130
30	Male	**31**	144	147	142	140
31	Male	**38**	161	124		
32	Male	**36**	195	136	129	
33	Male	**28**	227	136	128	
Minimum			77	53	47	53
25% Percentile			96	69	92	98
Median			**110**	**83**	**113**	**125**
75% Percentile			134	119.5	130.5	137
Maximum			227	147	142	161

Appendix 3: Maximum Resting Anal Canal Pressure (cmH_2O) In 20 Volunteers compared With 33 Patients with chronic anal fissures

VOLUNTEERS				PATIENTS
Sex	Age	MRP		
Female	23	46		77
Male	31	47		77
Male	25	54		83
Male	23	63		88
Female	24	67		88
Female	23	77		93
Female	33	82		93
Female	33	86		93
Female	38	87		98
Male	31	88		101
Female	24	88		101
Male	33	89		102
Male	23	98		103
Male	21	105		108
Female	35	111		109
Female	35	121		110
Male	21	130		110
Female	38	135		110
Male	33	136		112
Male	25	175		112
				115
				124
				128
				129
				131
				137
				141
				142
				143
				144
				161
				195
				227
Number of values		20		33
Minimum		46		77
25% Percentile		72		96
Median		**88**		**110**
75% Percentile		116		134
Maximum		175		227

Appendix 4: Maximum Resting Anal Canal Pressure (cmH_2O) after two months in 19 patients successfully treated after a 2 month course

		START	2 MONTHS
Sex	Age		
Female	40	77	55
Male	36	83	68
Male	81	88	75
Female	68	93	64
Female	28	93	75
Female	59	98	96
Female	29	103	61
Male	40	108	111
Female	27	109	66
Male	37	110	53
Male	27	110	83
Female	30	112	69
Female	42	115	119
Female	28	124	123
Male	28	131	81
Male	33	141	109
Male	29	143	125
Male	31	144	147
Male	36	195	136
Minimum		77	53
25% Percentile		93	66
Median		**110**	**81**
75% Percentile		131	119
Maximum		195	147

Appendix 5: Maximum Resting Anal Canal Pressure (cmH_2O) after two months in 14 patients who failed to heal after being treated after a 2 month course

		START	2 MONTHS
Sex	Age		
Male	38	77	83
Female	35	88	90
Female	29	93	103
Female	28	101	79
Female	27	101	68
Male	35	102	79
Female	35	110	107
Male	28	112	94
Male	33	128	56
Female	39	129	79
Female	27	137	120
Male	31	142	124
Male	38	161	124
Male	28	227	136
Minimum		77	56
25% Percentile		97	79
Median		**111**	**92**
75% Percentile		139.5	122
Maximum		227	136

Appendix 6: Maximum Resting Anal Canal Pressure (cmH_2O) in 19 patients who had anal spasm at presentation compared with 14 patients who did not

	SPASM	NO SPASM
	93	77
	93	77
	103	83
	108	88
	110	88
	110	93
	112	98
	112	101
	115	101
	124	102
	128	109
	131	110
	137	129
	141	142
	143	
	144	
	161	
	195	
	227	
Number of values	19	14
Minimum	93	77
25% Percentile	110	85.5
Median	**124**	**99**
75% Percentile	143	109.5
Maximum	227	142

Appendix 7: Maximum Resting Anal Canal Pressure (cmH_2O) in 22 patients who had sentinel piles at presentation compared with 11 patients who did not

	SENTINEL PILES	NO PILES
	112	98
	109	93
	93	88
	110	83
	195	103
	124	110
	77	108
	115	144
	141	88
	143	93
	131	77
	129	
	227	
	101	
	101	
	112	
	161	
	128	
	110	
	102	
	142	
	137	
Number of values	22	11
Minimum	77	77
25% Percentile	106	88
Median	**120**	**93**
75% Percentile	142	108
Maximum	227	144

Appendix 8: Maximum Resting Anal Canal Pressure (cmH_2O) in 12 patients who healed at two months after one course of topical diltiazem and remained healed at four months

		START	2 MONTHS	4 MONTHS
Sex	Age			
Male	81	88	75	77
Female	68	93	64	100
Male	40	108	111	110
Female	27	109	66	51
Male	37	110	53	120
Female	30	112	69	88
Female	42	115	119	132
Female	28	124	123	96
Male	28	131	81	115
Male	29	143	125	134
Male	31	144	147	142
Male	36	195	136	129
Minimum		88	53	51
25% Percentile		108.5	67.5	92
Median		**114**	**96**	**113**
75% Percentile		137	124	130.5
Maximum		195	147	142

Appendix 9: Maximum Resting Anal Canal Pressure (cmH_2O) in 8 patients who had not healed after two months of therapy with topical diltiazem. These patients received a second course of diltiazem. The results in bold italics represent the 2 patients that had not healed.

		START	2 MONTHS	4 MONTHS
Sex	Age			
Male	38	77	83	47
Female	29	93	103	101
Female	28	101	79	100
Male	35	102	79	117
Female	35	110	107	134
Male	***33***	***128***	***59***	***64***
Female	***39***	***129***	***79***	***135***
Male	28	227	136	128
Minimum		77	56	47
25% Percentile		97	79	82
Median		**106**	**81**	**109**
75% Percentile		128.5	105	131
Maximum		227	136	135

Appendix 10: Maximum Resting Anal Canal Pressure (cmH_2O) in 15 patients over a 6 month period with topical diltiazem. The results in bold italics represent the 2 patients that were unhealed at six months.

		START	2 MONTHS	4 MONTHS	6 MONTHS
Sex	Age				
Female	40	77	55		53
Male	81	88	75	77	76
Female	68	93	64	100	76
Female	29	93	103	101	125
Female	28	101	79	100	98
Male	35	102	79	117	161
Male	***40***	***108***	***111***	***110***	***134***
Male	37	110	53	120	137
Female	42	115	119	132	120
Female	28	124	123	96	137
Male	33	128	59	64	113
Female	***39***	***129***	***79***	***135***	***120***
Male	28	131	81	115	125
Male	29	143	125	134	130
Male	31	144	147	142	140
Minimum		77	53	64	53
25% Percentile		93	64	98	98
Median		**110**	**79**	**113**	**125**
75% Percentile		129	119	133	137
Maximum		144	147	142	161

NOVEL DELIVERY OF BOTULINUM TOXIN FOR TREATMENT OF ANAL FISSURES

Appendix 11: Pain scores (range 0, no pain –10, extreme pain) in patients with chronic anal fissures assessed over 12 weeks in 10 patents who received two 25-unit injections into the internal anal sphincter

Sex, age (years)	START	1 week	4 weeks	8 weeks	12 weeks
Female, 32	3	1	2	1	1
Female, 44	4	2	3	2	3
Male, 38	7	6	3	1	3
Female, 43	5	4	0	0	4
Female, 26	5	4	3	3	1
Male, 22	8	5	7	8	
Male, 71	6	3	4	4	3
Male, 42	6	4	2	0	0
Male, 52	7	6	7	5	6
Female, 28	8	8	8	8	7
Minimum	3	1	0	0	0
25% Percentile	4.5	2.5	2	0.5	1
Median	**6**	**4**	**3**	**2.5**	**3**
75% Percentile	7.5	6	7	6.5	5
Maximum	8	8	8	8	7

Appendix 12: Incontinence scores (range 0, perfect continence – 24, totally incontinent) in patients with chronic anal fissures assessed over 12 weeks in 10 patents who received two 25-unit injections into the internal anal sphincter

Sex, age(years)	START	1 week	4 weeks	8 weeks	12 weeks
Female, 32	1	4	6	3	6
Female, 44	9	10	14	15	15
Male, 38	0	0	8	3	0
Female, 43	7	9	4	6	4
Female, 26	0	0	0	0	0
Male, 22	4	4	8	4	
Male, 71	7	4	4	4	0
Male, 42	4	4	2	3	0
Male, 52	3	3	3	2	3
Female, 28	0	1	0	0	0
Minimum	0	0	0	0	0
25% Percentile	0	0.5	1	1	0
Median	**3.5**	**4**	**4**	**3**	**0**
75% Percentile	7	6.5	8	5	5
Maximum	9	10	14	15	15

Appendix 13: Maximum Resting Anal Canal Pressure (cmH_2O) in patients with chronic anal fissures assessed over 12 weeks in 10 patents who received two 25-unit injections into the internal anal sphincter

Sex, age (years)	START	1 week	4 weeks	8 weeks	12 weeks
Female, 32	105	87	80	89	95
Female, 44	77	68	81	62	91
Male, 38	170	123	105	107	107
Female, 43	91	85	104	110	108
Female, 26	80	62	76	82	74
Male, 22	129	105	105	101	
Male, 71	185	166	108	108	126
Male, 42	92	72	80	66	101
Male, 52	141	77	109	54	143
Female, 28	71	37	70	51	94
Minimum	71	37	70	51	74
25% Percentile	78.5	65	78	58	92.5
Median	**99**	**81**	**93**	**86**	**101**
75% Percentile	155.5	114	106.5	107.5	117
Maximum	185	166	109	110	143

SYMPATHETIC MODULATION OF THE INTERNAL ANAL SPHINCTER

Appendix 14: Hourly maximum Resting Anal Canal Pressure (cmH_2O) in 10 patients with chronic anal fissures and 10 volunteers who received an oral 20mg dose of indoramin over 3 hours

PATIENTS	START	1 hour	2 hours	3 hours
Sex, Age -years				
Female, 65	149	127	117	
Male, 35	227	169	114	115
Male, 38	135	98	71	92
Male, 37	195	145	140	120
Female, 30	126	67	92	91
Female, 33	198	129	111	98
Female, 28	119	65	71	57
Male, 40	117	57	100	85
Female, 36	110	78	88	58
Female, 29	94	76	61	57
Minimum	94	57	61	57
25% Percentile	113.5	66	71	57.5
Median	**130.5**	**88**	**96**	**91**
75% Percentile	196.5	137	115.5	106.5
Maximum	227	169	140	120

VOLUNTEERS	START	1 hour	2 hours	3 hours
Sex, Age -years				
Male, 33	136	117	96	75
Female, 35	121		114	112
Male, 25	54	40	46	56
Male, 23	63	49	47	39
Female, 24	67	63	46	33
Female, 38	87	59	81	66
Male, 21	130	92	82	76
Male, 31	88	63	51	38
Female, 23	46	36	47	50
Female, 33	82	78	59	48
Minimum	46	36	46	33
25% Percentile	58.5	44.5	46.5	38.5
Median	**84.5**	**63**	**55**	**53**
75% Percentile	125.5	85	89	75.5
Maximum	136	117	114	112

Appendix 15: Hourly maximum Resting Anal Canal Pressure (cmH_2O) in 10 patients with chronic anal fissures and 10 volunteers who received an oral 4mg dose of salbutamol over 3 hours

PATIENTS	START	1 hour	2 hours	3 hours
Sex, Age -years				
Female, 65	191	110	115	127
Male, 35	172	117	126	95
Male, 38	161	92	97	82
Male, 37	173	166	142	132
Female, 30	97	82	83	92
Female, 33	194	181	113	92
Female, 28	109	92	78	57
Male, 40	121	114	72	85
Female, 36	126	84	74	53
Female, 29	101	78	75	96
Minimum	97	78	72	53
25% Percentile	105	83	74.5	69.5
Median	**143.5**	**101**	**90**	**92**
75% Percentile	182	141.5	120.5	111.5
Maximum	194	181	142	132

VOLUNTEERS	START	1 hour	2 hours	3 hours
Sex, Age -years				
Male, 33	89	63	86	70
Female, 35	111	90	85	77
Male, 25	175	101	118	128
Male, 23	98	57	58	64
Female, 24	88	89	85	51
Female, 38	135	87	67	
Male, 21	105		92	68
Male, 31	47	44	56	47
Female, 23	77	53	47	34
Female, 33	86	65	64	71
Minimum	47	44	47	34
25% Percentile	81.5	55	57	49
Median	**93.5**	**65**	**76**	**68**
75% Percentile	123	89.5	89	74
Maximum	175	101	118	128

Appendix 16: Hourly pulse rate (/minute) in 10 patients with chronic anal fissures and 10 volunteers who received an oral 20mg dose of indoramin over 3 hours

PATIENTS	START	1 hour	2 hours	3 hours
Sex, Age -years				
Female, 65	72	72	68	
Male, 35	70	65	68	72
Male, 38	68	66	60	54
Male, 37	60	64	78	90
Female, 30	84	78	84	75
Female, 33	84	75	84	80
Female, 28	60	54	60	66
Male, 40	66	60	66	66
Female, 36	66	72	72	60
Female, 29	68	72	72	66
Minimum	60	54	60	54
25% Percentile	63	62	63	63
Median	**68**	**69**	**70**	**66**
75% Percentile	78	73.5	81	77.5
Maximum	84	78	84	90

VOLUNTEERS	START	1 hour	2 hours	3 hours
Sex, Age years				
Male, 33	68	64	68	64
Female, 35	66		60	60
Male, 25	84	72	68	72
Male, 23	78	78	84	76
Female, 24	60	66	66	66
Female, 38	60	60	84	84
Male, 21	66	72	78	78
Male, 31	76	84	84	84
Female, 23	70	72	74	70
Female, 33	66	72	70	74
Minimum	60	60	60	60
25% Percentile	63	65	67	65
Median	**67**	**72**	**72**	**73**
75% Percentile	77	75	84	81
Maximum	84	84	84	84

Appendix 17: Hourly pulse rate (/minute) in 10 patients with chronic anal fissures and 10 volunteers who received an oral 4mg dose of salbutamol over 3 hours

PATIENTS	START	1 hour	2 hours	3 hours
Sex, Age -years				
Female, 65	64	66	68	84
Male, 35	78	74	84	78
Male, 38	75	80	78	66
Male, 37	72	84	82	78
Female, 30	78	86	82	78
Female, 33	72	85	105	90
Female, 28	66	60	72	72
Male, 40	60	92	84	66
Female, 36	84	78	90	90
Female, 29	66	78	72	66
Minimum	60	60	68	66
25% Percentile	65	70	72	66
Median	**72**	**79**	**82**	**78**
75% Percentile	78	85.5	87	87
Maximum	84	92	105	90

VOLUNTEERS	START	1 hour	2 hours	3 hours
Sex, Age -years				
Male, 33	60	84	68	66
Female, 35	56	60	66	68
Male, 25	70	74	85	65
Male, 23	60	72	60	66
Female, 24	72	84	78	
Female, 38	84	76	80	82
Male, 21	72		60	66
Male, 31	84	84	90	84
Female, 23	60	72	78	78
Female, 33	68	80	66	72
Minimum	56	60	60	65
25% Percentile	60	72	63	66
Median	**69**	**76**	**73**	**68**
75% Percentile	78	84	82.5	80
Maximum	84	84	90	84

Appendix 18: Hourly systolic blood pressure (mmHg) in 10 patients with chronic anal fissures and 10 volunteers who received an oral 20mg dose of indoramin over 3 hours

PATIENTS	START	1 hour	2 hours	3 hours
Sex, Age -years	185	175	175	
Female, 65	120	118	120	118
Male, 35	128	126	122	114
Male, 38	122	118	118	126
Male, 37	138	134	126	128
Female, 30	142	150	144	144
Female, 33	108	102	110	112
Female, 28	129	128	122	114
Male, 40	112	115	114	112
Female, 36	108	118	118	108
Female, 29				
Minimum	108	102	110	108
25% Percentile	110	116.5	116	112
Median	**125**	**122**	**121**	**114**
75% Percentile	140	142	135	127
Maximum	185	175	175	144

VOLUNTEERS	START	1 hour	2 hours	3 hours
Sex, Age -years				
Male, 33	112	110	108	105
Female, 35	118		112	112
Male, 25	114	114	106	110
Male, 23	132	128	116	114
Female, 24	120	134	118	134
Female, 38	130	118	114	112
Male, 21	134	116	118	104
Male, 31	108	110	112	108
Female, 23	108	104	112	118
Female, 33	110	120	114	112
Minimum	108	104	106	104
25% Percentile	109	110	110	106.5
Median	**116**	**116**	**113**	**112**
75% Percentile	131	124	117	116
Maximum	134	134	118	134

Appendix 19: Hourly systolic blood pressure (mmHg) in 10 patients with chronic anal fissures and 10 volunteers who received an oral 4mg dose of salbutamol over 3 hours

PATIENTS	START	1 hour	2 hours	3 hours
Sex, Age -years	165	155	170	165
Female, 65	124	110	118	112
Male, 35	138	140	138	132
Male, 38	124	134	128	130
Male, 37	132	128	118	122
Female, 30	142	154	152	138
Female, 33	108	106	106	110
Female, 28	134	122	124	122
Male, 40	122	116	120	118
Female, 36	108	128	132	120
Female, 29				
Minimum	108	106	106	110
25% Percentile	115	113	118	115
Median	**128**	**128**	**126**	**122**
75% Percentile	140	147	145	135
Maximum	165	155	170	165

VOLUNTEERS	START	1 hour	2 hours	3 hours
Sex, Age -years				
Male, 33	110	122	118	112
Female, 35	108	111	120	115
Male, 25	114	122	124	116
Male, 23	124	120	114	132
Female, 24	132	130	128	
Female, 38	128	134	124	122
Male, 21	118		116	114
Male, 31	122	118	122	128
Female, 23	114	116	118	114
Female, 33	110	110	120	116
Minimum	108	110	114	112
25% Percentile	110	113.5	117	114
Median	**116**	**120**	**120**	**116**
75% Percentile	126	126	124	125
Maximum	132	134	128	132

Appendix 20: Maximum Resting Anal Canal Pressure (cmH_2O) in eight volunteers who received varying doses of topical indoramin (placebo, 10mg, 20mg, 30mg, 40mg)

Placebo

start	1 hour	3 hours	5 hours	7 hours
135	125	94	111	115
99	78	68	68	72
119	116	109	88	138
108	82	95	102	
115	120			
120	104	92	90	
74	72			
110	69	75	56	

10 mg

start	1 hour	3 hours	5 hours	7 hours
159	124	118	95	123
79	49	62		54
118	97	74		95
79	62	87		
104	104	125		
122	118	143		
78	60	67		
99	92	96	72	68

20 mg

start	1 hour	3 hours	5 hours	7 hours
182	122	109	117	105
79	52	77		
138	112		105	
83	85	88		
123	102	97	89	
113	73	88		
112	79	64	89	101
85	75	35	82	65

30mg

start	1 hour	3 hours	5 hours	7 hours
147	105	124	109	
87	67	51	63	94
126	97	127		
111	100	83	77	67
121	144	117		
73			63	72
98	78	80	78	
82	47	55	46	48

40mg

start	1 hour	3 hours	5 hours	7 hours
147	151	132		
77	58	83		
114	123	100		
99	90	89		
105	90	108		
120	96	106	84	92
90	89		68	84
92	82	96		

LABORATORY EXPERIMENTS

Appendix 21 : Effect of histamine (1x 10^{-5}, 3 x 10^{-5}, 1x 10^{-4}M) on the basal tone (g) of guinea-pig isolated IAS muscle strips.

	basal	new tone	significance
1x 10^{-4}M	0.21	2.5	
	0.04	0.83	
	0.27	1.87	
	0.44	2.4	$P = 0.014$
3 x 10^{-5}M	0.27	0.73	
	0.47	1.38	
	0.09	0.72	
	0.24	0.93	
	0.36	1.95	
	0.38	2.12	$P = 0.006$
1x 10^{-5}M	0.4	0.99	
	0.35	1.04	
	0.38	2.12	
	0.45	1.97	$P = 0.030$

Appendix 22: Effect of atropine (1 x 10^{-6} M) on basal tone (g) of guinea-pig isolated IAS muscle strips.

	basal	new tone	significance
1 x 10^{-6}M	0.36	0.41	
	0.48	0.61	
	0.07	0.05	
	0.4	0.47	
	0.17	0.22	
	0.04	0.06	$P = 0.058$

Appendix 23: Effect of methoxamine (1 x 10^{-4} M) on basal tone (g) of guinea-pig isolated IAS muscle strips.

	basal	new tone	significance
1 x 10^{-4}M	0.27	0.26	
	0.27	0.14	
	0.11	0.54	
	0.27	0.62	$P = 0.324$

Appendix 24: Effect of histamine on tone in 3 isolated porcine IAS muscle strips

[Histamine] M		Contraction (% of maximal response)		
$1x10^{-7}$		3.3	11.3	6.1
$3x10^{-7}$		5.7	24.1	11.7
$1x10^{-6}$		15.4	39.6	24.2
$3x10^{-6}$		25.2	51.9	35.2
$1x10^{-5}$		42.3	68.9	51.1
$3x10^{-5}$		64.2	82.1	62.5
$1x10^{-4}$		85.4	83.5	79.2
$3x10^{-4}$		88.6	92.0	86.7
$1x10^{-3}$		100	100	100

Appendix 25: Effect of phenylephrine on contractile response of porcine IAS muscle strips.

[Phenylephrine] M		Contraction (% of maximal response)						
$1x10^{-8}$		6.7	2.7	2.9	8.2	4.6	11.6	7.0
$3x10^{-8}$		9.0	4.4	5.2	17.4	4.6	14.2	8.7
$1x10^{-7}$		10.4	10.2	10.2	23.2	8.3	21.1	13.1
$3x10^{-7}$		23.9	20.7	16.1	28.9	11.0	22.4	17.8
$1x10^{-6}$		32.1	35.3	29.7	41.4	22.0	31.9	27.7
$3x10^{-6}$		53.7	59.7	49.5	80.3	37.6	45.3	44.0
$1x10^{-5}$		69.4	76.3	66.9	94.6	61.5	65.9	62.4
$3x10^{-5}$		84.3	93.9	83.6	98.5	72.5	84.1	86.3
$1x10^{-4}$		100.0	100.0	100.0	100.0	100.0	100.0	100.0

Appendix 26: Effect of isoprenaline on the relaxant response of porcine IAS muscle strips

[Isoprenaline] M		Relaxation (% of basal tone)				
$1x10^{-7}$		6.5	14.8			5.7
$1x10^{-6}$		4.4	17.6			3.3
$1x10^{-5}$		7.6	20.2	6.2	9.1	
$1x10^{-4}$		29.6	68.2	8.6	20.6	6.9
$1x10^{-3}$		22.0	48.3	6.1	14.1	4.7

Appendix 27: Effect of GTN on the relaxant response of porcine IAS muscle strips

[GTN] M		Relaxation (% of basal tone)							
$2.2x10^{-4}$		44.4	0.4	1.2	1.9	7.5	66.9	3.7	4.8
$6.6x10^{-4}$		56.9	0.4	7.6	6.4	3	73.4	3.5	22.1
$2.2x10^{-3}$		63.9	10.5	17.3	5.1	76.6	10.2	17.9	43.6
$2.2x10^{-4}$		11	3.4	58.1	3	18.2	26.2	1.3	1.5
$6.6x10^{-4}$		13.7	76.5	15.3	34.6	52.5	23.8	16.2	16.4
$2.2x10^{-3}$		7.7	5.3	76.8	22.1	58.4	51.1	25.5	29
$2.2x10^{-4}$		0.7	23	2.6	49.4	3	1.7	7.7	
$6.6x10^{-4}$		32.2	11	63.7	46	32.5	22.9		
$2.2x10^{-3}$		38.3	34.5	8.4	58.4	57.7	52.3	41.1	

Appendix 28: Effect of L-NAME on the acetylcholine induced relaxation of porcine IAS muscle strips.

		Relaxation (% of basal tone)						
Ach ($1x10^{-4M}$)		11.0	2.0	32.4	26.6	60.9	66.6	17.9
Ach ($1x10^{-4M}$) + L-Name ($1x10^{-4M}$)		0.6	1.1	0.6	0.3	0.7	1.8	2.1

Appendix 29: Effect of suramin on the ATP induced relaxation of porcine IAS muscle strips.

		Relaxation (% of basal tone)						
ATP ($1x10^{-3}$M)		24.0	5.2	3.4	4.6	24.2	8.7	21.3
ATP ($1x10^{-3M}$) + suramin ($3x10^{-4M}$)		23.6	7.2	17.2	14.8	33.2	15.9	30.1

Appendix 30: Effect of electrical field stimulation (EFS: 0.3 ms, 0.5-32 Hz, 60 V, train length 10 s, interval 110 s) on the relaxant amplitude of porcine IAS muscle strips.

Stimulation frequency (Hz)	Relaxant Amplitude (g)								
0.5	0.7	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1	1.2	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.1
2	2.0	0.2	0.2	0.1	0.1	0.3	0.5	0.2	0.1
4	2.7	0.4	0.3	0.2	0.1	0.4	1.0	0.3	0.3
8	2.9	0.9	0.5	0.2	0.1	0.6	1.6	0.3	0.5
16	3.2	1.3	0.5	0.2	0.2	0.6	1.9	0.3	0.5
32	2.8	1.4	0.5	0.2	0.1	0.6	1.9	0.2	0.6

Appendix 31: Effect of L-NAME ($1x10^{-4}$M) on the EFS-induced (EFS: 0.3 ms, 0.5-32 Hz, 60 V, train length 10 s, interval 110 s) relaxant amplitude of the porcine IAS muscle strips.

Stimulation frequency (Hz)	Relaxant Amplitude (g)			
0.5	0.2	0.1	0.1	0.1
1	0.2	0.2	0.2	0.1
2	0.2	0.2	0.2	0.1
4	0.2	0.1	0.3	0.2
8	0.2	0.2	0.5	0.3
16	0.2	0.2	0.6	0.3
32	0.3	0.2	0.7	0.4

Appendix 32: Effect of suramin ($3X10^{-4}$ M) on the relaxant response of porcine IAS muscle strips due to EFS (EFS: 0.3 ms, 0.5-32 Hz, 60 V, train length 10 s, interval 110 s).

Stimulation frequency (Hz)	control relaxation (g)				
0.5	2.0	1.7	5.5	3.2	5.3
1	6.2	2.5	8.9	4.0	4.1
2	9.9	5.0	14.1	5.6	3.3
4	16.5	7.5	21.6	8.6	6.5
8	24.0	9.9	26.3	12.5	6.9
16	21.8	10.2	29.1	12.1	4.1
32	20.4	8.6	28.8	13.1	5.3

Stimulation frequency (Hz)	with suramin (g)				
0.5	2.7	2.6	3.6	1.3	2.1
1	6.7	4.5	5.9	2.4	4.4
2	14.5	9.5	9.7	4.5	4.4
4	22.9	17.4	15.5	7.1	4.6
8	30.2	23.9	19.8	9.6	3.2
16	32.5	25.6	21.4	11.5	5.0
32	29.3	19.9	20.7	10.7	6.2

Appendix 33: Effect of prazosin ($1x10^{-7}$ M) on the relaxant response of porcine IAS muscle strips due to EFS (EFS: 0.3 ms, 0.5-32 Hz, 60 V, train length 10 s, interval 110 s).

Stimulation frequency (Hz)	control relaxation (g)					
0.5	4.2	14.1	12.8	14.3	3.3	10.8
1	5.9	15.7	8.6	21.4	10.2	19.3
2	11.8	26	10.7	31.7	15.9	26.5
4	17.4	29.1	17.1	40.5	26.3	38.6
8	19.9	30.7	21.4	45.2	34.4	39.8
16	8.5	9.4	22.5	46	36.6	18.1
32	3.8	7.9	25.1	42.9	35.8	9.6
	with prazosin (g)					
0.5	5.5	16.9	9.8	20.7	8.1	9.8
1	9.7	36.4	12.8	25.2	21.3	23.6
2	19.8	40.3	20.4	36	33.4	39
4	34.5	52.6	22.3	43.2	44.5	56.1
8	65.4	57.8	24.2	50.4	50	63.4
16	34.1	48.7	23	46.8	49.8	60.2
32	31.3	33.8	30.2	38.7	49.6	56.1

Appendix 34: Effect of prazosin ($1x10^{-6}$ M) on the histamine dose-response curve of porcine IAS muscle strips.

[Histamine] M	Contraction (% maximum)				with prazosin		
$1x10^{-7}$	1.0	22.4	8.7		10.8	6.8	1.5
$3x10^{-7}$	6.9	37.0	16.0		25.5	16.1	1.9
$1x10^{-6}$	8.8	54.2	32.0		33.3	21.4	10.2
$3x10^{-6}$	34.3	69.3	46.1		47.1	24.0	18.4
$1x10^{-5}$	59.8	84.9	67.0		59.8	42.7	35.9
$3x10^{-5}$	73.5	97.4	81.6		69.6	65.1	49.5
$1x10^{-4}$	100.0	100.0	100.0		78.4	82.3	68.9

Appendix 35: Effect of the α_1-antagonist indoramin ($1x10^{-7}$ M) on the phenylephrine dose-response curve in porcine IAS muscle strips.

[Phenylephrine] M	Tone (% of maximally raised tone)						
$1x10^{-8}$	6.7	2.7	2.9	8.2	4.6	11.6	7.0
$3x10^{-8}$	9.0	4.4	5.2	17.4	4.6	14.2	8.7
$1x10^{-7}$	10.4	10.2	10.2	23.2	8.3	21.1	13.1
$3x10^{-7}$	23.9	20.7	16.1	28.9	11.0	22.4	17.8
$1x10^{-6}$	32.1	35.3	29.7	41.4	22.0	31.9	27.7
$3x10^{-6}$	53.7	59.7	49.5	80.3	37.6	45.3	44.0
$1x10^{-5}$	69.4	76.3	66.9	94.6	61.5	65.9	62.4
$3x10^{-5}$	84.3	93.9	83.6	98.5	72.5	84.1	86.3
$1x10^{-4}$	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	with indoramin						
$1x10^{-8}$	0.7	0.3	0.3	0.7	3.7	1.3	0.6
$3x10^{-8}$	4.5	0.7	1.0	0.7	3.7	2.6	0.3
$1x10^{-7}$	4.5	3.7	1.6	0.2	5.5	5.6	0.6
$3x10^{-7}$	5.2	3.4	1.0	0.9	1.8	8.2	2.6
$1x10^{-6}$	11.2	7.1	2.9	0.4	4.6	20.7	8.5
$3x10^{-6}$	12.7	10.8	4.4	3.3	7.3	40.5	15.2
$1x10^{-5}$	17.9	26.8	13.8	7.4	14.7	67.2	33.5
$3x10^{-5}$	29.9	53.2	30.2	17.6	51.4	94.4	69.1
$1x10^{-4}$	36.6	73.6	50.3	29.7	68.8	112.9	97.1

Appendix 36: Effect of one-hour incubation of one unit of botulinum toxin (Botox®) on the acetylcholine (1x10-3M) induced relaxation of porcine IAS muscle strips.

	Relaxation (% basal tone)				
Ach	23.9	60.5	50.9	25.2	59.0
Ach + Botox	25.0	57.6	56.0	19.0	64.0

Appendix 37: Increase of the relaxant response of porcine internal anal sphincter (IAS) to electrical field stimulation (EFS: 0.3 ms, 16 Hz, 60 V, train length 10 s, interval 110 s) on tone raised by phenylephrine (1 μM-1 mM).

Tone (% maximal tone)	Relaxation (% maximal tone)		Tone (% maximal tone)	Relaxation (% maximal tone)		Tone (% maximal tone)	Relaxation (% maximal tone)
29.8	6.4		98.7	3.6		99.0	10.3
26.1	6.0		91.6	3.4		97.2	8.4
34.2	10.7		93.1	3.4		94.9	8.7
45.7	12.1		38.2	3.3		92.1	8.3
61.1	13.6		43.4	4.7		60.3	5.6
74.9	13.6		48.6	5.5		65.3	6.1
91.7	12.2		49.1	7.6		68.3	5.9
98.2	10.7		58.1	9.2		77.3	6.9
100.0	9.4		64.7	11.8		83.5	7.5
33.8	7.4		69.2	12.6		90.7	7.2
36.3	4.2		71.1	12.6		93.1	8.3
45.8	6.0		78.7	12.1		97.3	8.0
51.8	7.0		88.9	14.5		95.5	7.2
61.3	10.6		90.5	12.6		100.0	8.8
68.3	13.0		94.1	14.5		81.2	18.8
74.3	16.2		98.8	13.7		86.8	24.8
75.4	17.6		100.0	12.3		86.3	22.6
78.5	16.9		17.6	6.7		93.6	25.6
83.1	19.4		20.6	6.8		95.7	24.4
90.1	21.8		22.7	7.6		99.1	26.1
94.7	24.6		22.6	7.5		96.6	26.1
97.5	25.7		31.4	8.9		100.0	28.2
97.9	25.0		38.9	10.6		97.9	26.9
100.0	26.1		39.7	10.0		100.0	26.1
99.6	26.1		54.8	14.5		42.9	16.8
15.7	2.1		63.7	15.2		46.8	16.1
15.0	1.7		69.4	12.2		55.5	21.0
14.2	1.1		85.6	15.1		60.3	22.9
15.0	1.3		84.5	13.3		61.6	26.1
65.2	2.6		85.8	12.1		64.5	22.6
68.2	3.0		92.0	11.4		73.2	27.4
76.6	2.6		95.6	9.6		79.7	27.7
82.1	2.8		96.5	7.8		89.4	27.1
87.7	2.6		96.7	8.3		91.9	25.2
92.3	3.2		100.0	9.1		96.1	25.5
95.1	3.2		81.9	15.2		98.7	26.5
95.7	3.0		96.1	19.4		95.8	25.5
99.1	3.2		98.4	18.8		100.0	26.8
99.6	3.6		97.7	18.4		63.8	8.1
100.0	3.4		100.0	20.4		75.4	12.3
			81.6	16.2		81.2	14.6

Appendix 38: Residual tone of response to electrical field stimulation (EFS: 0.3 ms, 16 Hz, 60 V, train length 10 s, interval 110 s) on tone raised by phenylephrine (1 μM-1 mM) in the porcine internal anal sphincter (IAS).

Tone (% maximal tone)	Residual tone (% maximal)		Tone (% maximal tone)	Residual tone (% maximal)		Tone (% maximal tone)	Residual tone (% maximal)
29.8	23.5		98.7	95.1		99.0	88.7
26.1	20.1		91.6	88.2		97.2	88.8
34.2	23.5		93.1	89.7		94.9	86.2
45.7	33.7		38.2	34.8		92.1	83.8
61.1	47.5		43.4	38.6		60.3	54.7
74.9	61.3		48.6	43.1		65.3	59.2
91.7	79.5		49.1	41.5		68.3	62.4
98.2	87.5		58.1	48.8		77.3	70.4
100.0	90.6		64.7	52.8		83.5	76.0
33.8	26.4		69.2	56.6		90.7	83.5
36.3	32.0		71.1	58.5		93.1	84.8
45.8	39.8		78.7	66.6		97.3	89.3
51.8	44.7		88.9	74.4		95.5	88.3
61.3	50.7		90.5	78.0		100.0	91.2
68.3	55.3		94.1	79.6		81.2	62.4
74.3	58.1		98.8	85.1		86.8	62.0
75.4	57.7		100.0	87.7		86.3	63.7
78.5	61.6		17.6	10.9		93.6	67.9
83.1	63.7		20.6	13.8		95.7	71.4
90.1	68.3		22.7	15.1		99.1	73.1
94.7	70.1		22.6	15.1		96.6	70.5
97.5	71.8		31.4	22.5		100.0	71.8
97.9	72.9		38.9	28.3		97.9	70.9
100.0	73.9		39.7	29.8		100.0	73.9
99.6	73.6		54.8	40.2		42.9	26.1
15.7	13.6		63.7	48.4		46.8	30.6
15.0	13.3		69.4	57.2		55.5	34.5
14.2	13.1		85.6	70.4		60.3	37.4
15.0	13.6		84.5	71.3		61.6	35.5
65.2	62.6		85.8	73.7		64.5	41.9
68.2	65.2		92.0	80.6		73.2	45.8
76.6	74.0		95.6	86.0		79.7	51.9
82.1	79.3		96.5	88.7		89.4	62.3
87.7	85.0		96.7	88.4		91.9	66.8
92.3	89.2		100.0	90.9		96.1	70.6
95.1	92.0		63.8	55.7		98.7	72.3
95.7	92.7		75.4	63.1		95.8	70.3
99.1	95.9		81.2	66.7		100.0	73.2
99.6	96.1		81.6	65.4			
100.0	96.6		81.9	66.7			
97.7	79.3		96.1	76.7			
100.0	79.6		98.4	79.6			

Appendix 39: Relationship of tone (% maximum) to residual tone (% maximum) for porcine internal anal sphincter (IAS) relaxation induced by glyceryl trinitrate (GTN: $2.2x10^{-4}$ M, $6.6x10^{-4}$ M, $2.2x10^{-3}$ M).

$2.2x10^{-4}$ M			$6.6x10^{-4}$ M			$2.2x10^{-3}$ M	
Tone (% maximum)	Residual tone (% maximal)		Tone (% maximum)	Residual tone (% maximal)		Tone (% maximum)	Residual tone (% maximal)
17.5	9.8		17.5	7.6		17.5	6.3
56.6	55.6		56.6	55.6		56.6	50
100	98.8		100	92.4		100	82.7
18	17.6		18	16.8		100	95.4
100	83.5		100	89.3		22.5	5.3
22.5	7.5		22.5	6		69.1	60.3
69.1	64.7		69.1	64.9		28.5	23.4
100	95.2		100	77.9		100	56.4
33.3	29.7		33.3	28.8		65.3	60.3
100	91.8		44.9	10.6		100	95
44.9	18.8		82.1	69.6		44.9	10.4
82.1	79.7		100	65.4		82.1	64
100	80.9		52.4	24.9		100	41.6
52.4	38.7		88.8	67.7		52.4	25.7
88.8	87.7		80.3	68		88.8	66.2
80.3	74.7		100	83.6		80.3	53.9
100	99.3		47	31.9		100	71.7
47	36.2		100	90.8		47	30.8
100	81.1		30.6	8.6		100	84.3
30.6	11.9		91.1	49.2		30.6	9.8
91.1	88.4		72.5	48.9		91.1	38.5
72.5	71.3		100	82.6		72.5	34.6
100	98.8					100	63

Appendix 40: Relationship of tone (% maximum) to residual tone (% maximum) for porcine internal anal sphincter (IAS) relaxation induced by diltiazem ($1x10^{-4}$ M).

Tone (% maximum)	Residual tone (% maximal)
100.0	86.2
93.1	86.2
72.4	62.9
69.8	64.7
89.7	87.1
100.0	74.0
44.6	37.3
27.9	24.5
52.8	45.6
88.2	86.6
90.0	78.0
97.4	94.4
100.0	97.0
81.6	69.6
100.0	84.3
82.9	77.4
85.7	81.1
83.9	81.6
82.0	69.1
100.0	82.9
83.9	80.6
84.3	80.2
60.1	54.0
84.8	77.8
78.8	74.7
92.4	88.4
100.0	93.9

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